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Abstracts

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Abstracts

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International Congress of Human Genetics 2011
Montreal, Canada
October 11-15, 2011
The ESHG is a founding member of the IFHGS - International Federation of Human Genetics Societies

European Human Genetics Conference 2012
June 2 - 5, 2012
Nürnberg, Germany

European Human Genetics Conference 2013
Paris, France
June 8 - 11, 2013

Spoken Presentations

Posters

Abstracts marked with * and a presentation number highlighted in gray are talks of Young investigator Candidates

Abstracts marked with ** and a presentation number highlighted in blue are ESHG Poster Award Candidates

Abstracts marked with *** and a presentation number highlighted in green are within the Best Scored Posters

PL1.1
How next generation sequencing changes the practice of medicine

***H. Brunner**;*
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Now that massively parallel (or next generation; NGS) sequencing has become a reality, it is time to ask the question whether this technology will change the way we practice medicine in the clinic. While true costs that include personnel, bioinformatics support and equipment will be considerably higher, we may expect that the costs for sequencing 50-150 genes should not exceed 1000 Euro. This will allow the creation of diagnostic panels of for many clinical situations (muscular dystrophy; neurodegenerative disorders; blindness, deafness, cardiomyopathy; long QT syndrome; Noonan and related conditions etc). This will largely replace the testing of single genes, or of several genes in succession as is now customary. The projected cost of consumables for sequencing all 21000 human genes (the exome) using the SOLID platform is 2000 Euros. This could be used to test for instance to detect ***de novo*** changes in mental retardation patients. We may predict the following:

• Diagnostic testing of genes will move to the front end of the diagnostic process in many clinical situations. When a patient is seen in the outpatient clinic, an exome may already be done.

• Clinical geneticists should themselves aim to be involved in the diagnostic process from the start. This argues for multidisciplinary diagnostic clinics

• Clinical diagnostic skills (in dysmorphology, or other) will become less important and understanding genes and their functions in terms of the human organism becomes a critical skill.

• Numerous patients will be diagnosed with unique (new) genetic conditions. This calls for geneticists to participate and perhaps organize specialist clinics for (very) rare diseases. Geneticists should develop skills that allow them to feel comfortable in their role as the ultimate expert on a subset of these very rare conditions.

• Clinical geneticists should consider setting up services for the public that deal with incidental and minor findings on exome analysis. This may well be in the shape of e-consults rather than of formal outpatient clinics.

• The counseling element of clinical genetics (what am I to do with my life given my genetic profile and risks) remains essentially unchanged.

PL1.2
Unraveling the Dutch genome in health and disease

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No abstract received as per date of production. Please see www.eshg.org/eshg2011 for possible updates.

PL1.3
LGR5 stem cells in health and disease

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No abstract received as per date of production. Please see www.eshg.org/eshg2011 for possible updates.

PL2.1*
De novo balanced chromosomal rearrangements have high risk of neurodevelopmental and psychiatric disorders.

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Carriers of prenatally detected *de novo* balanced reciprocal translocations or inversions are believed to have 6-9% risk of congenital malformations and/or developmental delay. However, systematic data regarding late-onset disorders are lacking.

A cohort (N=41) of unselected carriers, that were detected prenatally in the period 1970-2008, was identified in the Danish Cytogenetic Central Register. Using information from national medical registries, we compared morbidity in the cohort to that of a 5:1 matched control group (N=205). Carriers were offered clinical re-examination, analysis with Affymetrix SNP 6.0 Array, and breakpoint mapping with Next Generation Sequencing (NGS) Mate-pair Analysis.

We observed no serious congenital malformations in the cohort. However, the risk of neurodevelopmental and/or psychiatric disorders, including mental retardation, learning disabilities, attention and/or behavioral disorders, autism spectrum disorders, and mood disorders, was significantly elevated (19.5% vs. 8.3% among the controls, p=0.04). Analysis for copy number variants >100 kb did not provide prognostic information about late-onset disorders. So far Mate-pair sequencing indicate that many affected carriers have complex rearrangements and/or rearrangements that truncate brain expressed genes, including genes known to be involved in neurodevelopmental disorders.

Our study demonstrated that prenatally detected *de novo* balanced chromosomal rearrangements have high risk of late-onset neurodevelopmental and/or psychiatric disorders but are not associated with congenital malformations. The breakpoint mapping, which will be completed in March 2011, will reveal whether NGS can improve the counseling dilemma associated with prenatally detected apparently balanced *de novo* chromosomal rearrangements.

PL2.2
Development and validation of non-invasive prenatal diagnostic test for trisomy 21

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The development of a Non-Invasive Prenatal Diagnostic (NIPD) test for Down syndrome (Trisomy 21) has become one of the most interesting fields in the prenatal world of the last decade. Extensive investigation of epigenetic differences between the mother and the fetus has led to the identification of Differentially Methylated Regions (DMRs) which have the potential to be used in the development of NIPD test for Trisomy 21 (Papageorgiou et al., AJP, 2009).In this study, we have been using a subset of our previously identified DMRs to develop a non-invasive prenatal diagnostic test for Trisomy 21 using the Methylated DNA Immunoprecipitation (MeDIP) methodology in combination with Real-time qPCR. The statistical significance of the 12 selected DMRs was evaluated using the Mann-Witney U test and selection of the best DMRs was followed.

We hereby present a strategy to achieve non-invasive fetal chromosome dosage assessment through the analysis of fetal specific DMRs. We achieved non-invasive prenatal detection of Trisomy 21 by determining the methylation ratio of normal and Trisomy 21 cases for each tested fetal specific DMR present in maternal peripheral blood, followed by further statistical analysis. The application of the above fetal specific methylation ratio approach provided with 100% accuracy the correct diagnosis of 66 normal and 34 Trisomy 21 pregnancies.

We present in this study the successful development and validation of a low cost, fast, sensitive and universal, non-invasive prenatal diagnostic test for Trisomy 21 which can be potentially implemented in diagnostic laboratories.

PL2.3*
mRNA-Seq analysis of monozygotic twins discordant for Trisomy 21 reveals large chromosomal domains of gene expression dysregulation

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Trisomy 21 (T21) is the most widely studied model of aneuploidy. It is likely that the majority of the phenotypes are related to alterations of gene expression. In this study, we investigated the perturbations of gene expression in fibroblasts derived from a pair of monozygotic twins discordant for T21. The use of these samples eliminates the bias of genome variability and most of transcriptome differences observed are likely to be related to the supernumerary chromosome 21.
The transcriptome was studied by mRNA-Seq; more than 200 million 100bp paired-end reads were generated and mapped using MAQ and GEM (Ribeca et al, unpublished). The expression level of 15'000 protein-coding genes was compared between the discordant twins. On average, we found that 4% of the genes are differentially expressed. Surprisingly, for most of the chromosomes, we identified large chromosomal regions that are either up- or downregulated in the trisomic twin. This raised the hypothesis that differential expression may be organized in domains throughout the genome. The same domains of gene expression dysregulation have also been found when we compared the transcriptomes of induced pluripotent stem (iPS) cells derived from the same twins fibroblasts, indicating that iPS cells retain a specific memory of their origin. Control comparisons of fibroblasts between normal monozygotic twins showed some transcriptional differences likely due to the contribution of stochastic, technical and environmental factors.
Our study reveals a unique finding in genome regulation of T21 nuclei and may be important for the molecular pathogenesis of whole and partial chromosome aneuploidies.

PL2.4
Next-generation sequencing in >240 families with X-linked intellectual disability

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X-linked intellectual disability (XLID) is a clinically and genetically heterogeneous condition. It accounts for 10% of all forms of intellectual disability. To date, mutations in >90 genes have been implicated in this disorder, yet in the majority of families, the underlying molecular cause is still unknown. We performed Next Generation Sequencing (NGS) for mutation detection in >240 XLID families that had been recruited by the European MRX Consortium and associated groups. In total, we detected potentially XLID causing mutations in up to 45% of our cohort. Only 28% of the families carried mutations in the >90 established XLID genes, which may be due to the fact that prior to NGS, many of the families had undergone pre-screening for mutations in known XLID genes. Unique truncating and/or novel perfectly co-segregating, functionally plausible missense mutations with high pathogenicity scores were detected in 20 genes that had not been implicated in XLID before. Numerous of these novel genes play a role in the regulation of transcription, while others are involved in ubiquitin-mediated protein degradation or in synaptic function, and many interact with ID genes that are already known. To our knowledge, this is the largest study ever into the molecular causes of XLID and the first one employing NGS to elucidate monogenic disorders in a systematic manner. It illustrates that in spite of a previous, Sanger-sequencing-based 'drain the pond' screening effort (Tarpey et al, Nature Genetics, 2009), the pond of unknown XLID genes is not empty yet.
Supported by BMBF, Max-Planck-Society, EU.

PL2.5*
Identification of the gene underlying Congenital Short Bowel Syndrome, pointing to its major role in intestinal development

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Congenital Short Bowel Syndrome is an autosomal recessive disorder characterized by substantial shortening of the small intestine and by intestinal malrotation. Until recently, nothing was known about the genetic cause. Homozygosity mapping was performed using 610K SNP arrays of Illumina on five patients of four different families, including one consanguineous family with two affected siblings and one unaffected child. We found an overlapping homozygous region in four of the five patients. In this region a homozygous deletion concerning one exon of a gene encoding a tight-junction protein, was detected in one of the patients. Furthermore, a homozygous deletion in the first intron was detected in the affected siblings of the consanguineous family, this deletion co-segregates with the disease phenotype in this family. Sequencing of the gene in three other patients resulted in the identification of additional mutations: one patient proved to have a heterozygous frameshift mutation and a heterozygous splice site mutation, whereas two other patients were

homozygous for a nonsense mutation and a missense mutation, respectively. The gene is expressed in the intestine of human embryos throughout development. The missense mutation abrogated the normal localization of the encoded protein at the cell membrane. Knock-down experiments in zebrafish resulted in general developmental defects, including shortening of the intestine and absence of goblet cells, which are characteristic for the mid-intestine. Therefore, loss-of-function of the identified gene leads to Congenital Short Bowel Syndrome, likely by interfering with tight-junction formation, with intestinal development and with gut length determination.

PL2.6*

HLA-A*3101 and Carbamazepine-Induced Hypersensitivity Reactions in Europeans

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Carbamazepine (CBZ) is the most commonly prescribed anti-epileptic drug. However its use can cause various forms of subcutaneous adverse reactions ranging from maculopapular exanthema (MPE) to Stevens-Johnson syndrome (SJS), a life-threatening skin reaction. A strong correlation has been demonstrated between CBZ-induced SJS and HLA-B*1502 in Asian populations however as HLA-B*1502 is largely absent in European populations, the test is not applicable in Europeans.

We investigated whether genetic variants, particularly within the HLA locus, play a role in susceptibility to CBZ-induced adverse reactions. We performed a genome-wide association study of 22 participants with CBZ-induced hypersensitivity syndrome (HSS), 43 participants with CBZ-induced MPE and 3958 population controls, all of European descent. We replicated the study with an independent set of participants (n=145) with CBZ-induced MPE and SJS.

HLA-A*3101 strongly associated with both HSS (3.5x10-8) and MPE (1.1x10-6) in two independent association studies. Follow-up genotyping of HLA-A*3101 in additional cases and controls confirmed HLA-A*3101 as a risk factor for HSS (OR 12.4; 95% CI 1.3 to 120.4), MPE (OR 8.3; 95% CI 3.6 to 19.4), and SJS (OR 25.9; 95% CI 4.9 to 116.2).

We report for the first time in participants of European ancestry, a strong association between HLA-A*3101 and CBZ-induced hypersensitivity. This finding has immediate clinical relevance in the care of epilepsy patients. The presence of HLA-A*3101 increases the probability of developing hypersensitivity from 5% to 26%, while its absence reduces the risk to 3.8%. This work provides the foundation for genetic testing of HLA-A*3101 for all prospective CBZ users.

PL3.1

The personal genomes of individuals with extreme phenotypes: Cardiac repolarization & sudden cardiac death

A. Chakravarti;

McKusick - Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States.

Although the genome (actually, two genomes) of specific individuals are now being sequenced there is great uncertainty regarding what phenotypic predictions we can make from the sequence itself. Accurate phenotypic predictions may be impossible unless we are more educated about the genetic architecture of human disease and the contributions of epigenetic and environmental effects. One exception to this expectation is perhaps phenotypic prediction in individuals with a rare susceptibility allele or an excess of common susceptibility variants.

Sudden cardiac death, arising from dysregulated cardiac repolarization, is a hallmark of the long QT syndrome and extreme elevations of the QT interval. A variety of human genetic studies by many investigators, including us, have identified over 30 genes contributing to inter-individual variation in the QT interval. We will summarize the current genetic and genomic knowledge in this area, and the study of families and population cohorts, to evaluate whether phenotypic prediction of sudden cardiac death is possible at least for a defined segment of the population.

PL3.2

Focus on genomic personalised medicine

C. Bustamante;

Bio-X , Stanford School of Medicine, Stanford, CA, United States.

No abstract received as per date of production. Please see www.eshg.org/eshg2011 for possible updates.

PL3.3

The genetics of type 2 diabetes: crossing the translational bridge.

M. McCarthy[1,2];

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Until recently, progress in identification of the genetic variants influencing predisposition to common forms of diabetes and obesity has been slow. However, advances have transformed the situation, with researchers now able to undertake well-powered surveys of genetic variation across the genome.

For T2D, genome-wide association studies have extended the number of loci harbouring common variants implicated in diabetes-susceptibility beyond 40. Most of these seem to impact on beta-cell function and there is evidence that cell cycle regulation pathways are overrepresented. However, even in combination, these variants account for only around 10% of the genetic predisposition to T2D.

Genetic diagnostics plays an increasingly important role in the diagnosis of relatively rare monogenic forms of diabetes and obesity and provides a molecular basis for accurate prognostication and therapeutic optimisation. In contrast, the modest effect of the common variants thus far implicated in common forms of diabetes and obesity has precluded similar clinical applications. Indeed, the principal translational justification for genetic discovery efforts lies in explaining the mysteries of disease pathogenesis: there have been numerous advances in this respect, though much more remains to be done.

The large proportion of heritability and familiality not explained by the GWA approach (which has surveyed only common SNP-based variation) raises questions about the basis of the "missing" genetic variance. The contribution to disease risk of low-frequency and rare variants is being enumerated through large-scale next-generation sequencing studies. Discovery of low frequency penetrant alleles influencing T2D risk (and/or risk of obesity and other metabolic and cardiovascular traits) will not only provide important insights into disease pathogenesis, but also powerful reagents to support functional and physiological studies.

PL4.1
Mendel Lecture
E. H. Blackburn;
Department of Biochemistry and Biophysics, The University of California, San Francisco, CA, United States.
No abstract received as per date of production. Please see www.eshg.org/eshg2011 for possible updates.

PL5.1
ESHG Award Lecture: Genetics in health care: about to make a (big) difference
G. J. B. van Ommen;
Dep.of Human Genetics, Center for Human and Clinical Genetics, University Medical Center, Leiden, Netherlands.
No abstract received as per date of production. Please see www.eshg.org/eshg2011 for possible updates.

S01.1
Controlling gene expression by the regulation of chromatin compaction in the nucleus

***W. Bickmore**;*
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It is widely appreciated that histone modifications correlate to gene expression states and can, in some cases, directly impact on gene expression and repression. But, our understanding of chromatin states beyond the level of the nucleosome itself is rudimentary and so there has been little exploration of how these levels of chromatin structure might contribute to the regulation of gene expression.
Important and well studied regulators of gene expression during development are the polycomb repressive complexes (PRCs). These complexes have also been implicated in human cancers and their histone modifying activities have been studied extensively. However, we show that polycomb repressive complexes act at the level of chromatin compaction in the nucleus and we go on to demonstrate that the ability of the PRC1 complex to compact chromatin, and to repress gene expression is not dependent on its histone ubiquitination activity.

S01.2
Transcription and the three-dimensional genome

***V. Corces**;*
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S01.3
Topography of nuclear functions studied by high-resolution microscopy

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Recent developments of light optical nanoscopy with resolution beyond the classical Abbe limit (1) have supplemented electron microscopic approaches (2,3) to study the structure of individual CTs in space and time and their spatial relationships to non-chromatin domains (4). The results of these studies support the chromosome territory-interchromatin compartment (CT-IC) model of nuclear architecture (5): CTs are built up from a network of interconnected chromatin domains with a DNA-content in the order of 1 Mb (~1Mb CDs) . Their structure has not yet been resolved, but we argue that these domains are built up from a series of more or less compacted chromatin loop domains with a DNA content in the order of 100 kb (~100 kb CDs). Moreover, several ~1Mb CDs may form larger chromatin clusters. The IC forms a 3D network of channels and larger, DNA free IC-lacunas (width > 400 nm). It starts at the nuclear pores and pervades the nuclear interior expanding both between CTs and within CTs. Accordingly, CTs may be compared with sponges built up from 3D interconnected chromatin domains and the pervading IC channels. A layer of decondensed chromatin, termed the perichromatin region (PR), covers the more compact interior of ~1 Mb-CDs and constitutes the nuclear compartment for transcription and DNA-replication (1, 3).
(1) Markaki, Y., et al. *Cold Spring Harb Sym Quant. Biol. 75* (2011) [Epub ahead of print].
(2) Rouquette, J et al. *Chromosome Res 17* (2009) 801-810.
(3) Niedojadlo, J. et al. *Exp. Cell Res 317* (2011) 433-444.
(4) Rouquette, J., et al. , *Int. Rev Cell Mol Biol 282* (2010) 1-90.
(5) Cremer, T. and Cremer, M. *Cold Spring Harb Perspect Biol 2* (2010) a003889.

S02.1
Generation of organs from pluripotent stem cells

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S02.2
Modeling rare blood diseases with induced pluripotent stem cells

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Genetic mutations associated with blood diseases are being discovered at an unprecedented rate. To develop new treatments, we must determine how these mutations cause defects in hematopoietic development and cell function. The advent of "direct reprogramming" technology allows us to revert a patient's skin or blood cells to an embryonic state, yielding "induced pluripotent stem" (iPS) cells. iPS cells carrying patient mutations can in turn be differentiated into numerous specific tissue types to examine pathogenesis in a developmental context. iPS technology is well suited for modeling human genetic blood disorders (e.g. dyskeratosis congenita, Pearson marrow pancreas syndrome, Shwachman Diamond syndrome) that have been a challenge to study using engineered mice or primary patient cells. Work-to-date creating iPS cells from patients with bone marrow failure syndromes is providing insights into disease mechanisms and stem cell biology, and prospects for therapy.

S02.3
Induction of Pluripotency

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The mammalian germline comprises two principal parts: the inner cell mass and epiblast, containing pluripotent cells, and the germ cell lineage, hosting unipotent cells. Several pluripotent stem cell types have been derived from the first part. Embryonic stem (ES) cells, derived from preimplantation embryos, comprise at least two populations of cells with divergent states of pluripotency. In addition, epiblast stem cells (EpiSCs), derived from postimplantantion embryos, also do not comprise a uniform cellular population, as we've shown the presence of at least two EpiSC types: one resembling epiblast tissue of early and the other of late pregastrulation embryos.
A current aim in cell and developmental biology is to program cells at will. The first step in converting a given cell into another cell is through achievement of a pluripotent stem cell state that resembles that of ES cells. To date, somatic cells need to be pushed to a pluripotent state by the introduction of exogenous factors, mostly transcription factors. Reprogramming of mouse and human somatic cells into pluripotent stem cells, designated as induced pluripotent stem (iPS) cells, was first described in 2006 using fibroblasts as the somatic cell source and initially requiring introduction of the virally-expressed transcription factor quartet of Oct4, Sox2, c-Myc, and Klf4. As we have recently shown, induced EpiSCs (iEpiSCs) can be obtained by directly reprogramming somatic cells with the quartet under EpiSC culture conditions. We previously reported that Oct4 alone is sufficient to directly reprogram adult mouse and human fetal neural stem cells (NSCs) into iPS cells, thus highlighting the crucial role played by Oct4 in the process of reprogramming.
In contrast to the recent reprogramming of somatic cells, induction of pluripotency in primordial germ cells (PGCs) was accomplished 20 years ago by the mere modulation of the culture conditions. Recently, we converted adult germline stem cells (GSCs) into germline-derived pluripotent stem (gPS) cells. GSCs are unipotent cells of the testis that are capable of not only self-renewing, but also giving rise to sperm. Like ES cells, GSCs exhibit significant levels of Oct4 and Klf4, but low endogenous expression Sox2 and c-Myc. To better understand the reprogramming process, we sought to identify factors that mediate reprogramming at higher efficiency. We established an assay based on *Oct4* reactivation to screen nuclear fractions from extracts of pluripotent cells. **BAF chromatin remodeling complexes containing the Brg1 protein have been shown to be not only** essential for early embryonic development, but also, as we have previously shown, paramount in enhancing the efficiency of reprogramming somatic cells to pluripotency mediated by the quartett. As knockdown of Brg1 leads to differentiation of ES cells, we investigated the early effect of Brg1 knockdown by assessing the impact of RNA interference on the expression levels of key pluripotency factors. We show that Brg1

knockdown leads to an immediate alteration in *Oct4, Sox2*, and *Nanog* expression. More specifically, *Sox2* expression is downregulated, while *Nanog* expression is upregulated. *Oct4* expression is immediately upregulated, perhaps triggering ES cell differentiation. Our data suggest that Brg1 plays an important role in regulating Oct4 expression, which is instrumental in maintaining cells in a pluripotent state.

S03.1
Dissecting regulatory variation in human genomes using RNA sequencing

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Our ability to understand and predict complex human traits is being enhanced by our ability to uncover the effects of genetic variation on cellular state. Specifically, by identifying the genetic variants which influence the expression of particular genes it is likely we are also uncovering those variants which inform various human conditions. Now, with advances in RNA sequencing (RNA-Seq), we can better interrogate transcriptome complexity; this provides us with the ability to better understand the repertoire and abundance of transcripts in multiple cell types. Furthermore, with the increasing availability of individual genomes we have the resolution to integrate rare and common as well as small and large variants into a more complete model of association with the aim of pinpointing specific causal variants. I will discuss what we are learning and how genetic studies of gene expression are evolving using RNA-Seq and population genome sequencing.

S03.2
Exploring the invisible world using metagenomics: systemic analysis of the ecosystem ‚human gut'

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Environmental sequencing (metagenomics) is uncovering genomic parts lists of various microbial communities, but our understanding of their functioning remains limited. I will briefly discuss the status of this young field (Raes and Bork, 2008), in which due to utilization of NGS data and ambitions increase exponentially. I will illustrate the developments using the example of the human gut, where recently NGS has been demonstrated to provide an entry a new dimension in analysis (Qin et al., Nature 2010). New biological discoveries, like the identification of a stratification of microbial communities in the human population dubbed enterotypes (Arumugam et al., 2011) come along with big hopes for microbial markers in various diseases with considerable diagnostic potential.

Raes J and Bork B, Nat Rev Microbiol. 2008 Sep;6(9):693-9.
Qin J et al., Nature. 2010 Mar 4;464(7285):59-65.
Arumugam et al., Nature 2011, in press

S03.3
Sequencing Thousands of Human Genomes

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Identifying and characterizing the genetic variants that affect human traits is one of the central objectives of human genetics. Ultimately, this aim will be achieved by examining the relationship between interesting traits and the whole genome sequences of many individuals. Whole genome re-sequencing of thousands of individuals is not yet feasible, but advances in laboratory methods (for example, to enable the genotyping of thousands of individuals at hundreds of thousands of SNP sites) and in statistical methodology (for example, to enable accurate correction for population stratification and genotype imputation) have resulted in substantial progress in our understanding of complex disease biology.

Here, we discuss practical study designs for the first generation of whole genome sequencing studies. These will enable the examination of 1,000s of individuals at >15 million of polymorphic sites. These studies will be enabled by continuing advances in laboratory technology and statistical methods and should further refine our understanding of complex disease genetics. I illustrate the possibilities both with simulation and with results from ongoing studies.

S04.1
Joubert Syndrome: impaired cilia and Shh dependent cerebellar development

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Joubert Syndrome (JS) is a multisystemic disorder characterized by a complex cerebellum/brainstem malformation where the most striking feature is a severe cerebellar vermis defect ranging from hypoplasia to complete aplasia. JS belong to a group of rare genetically heterogeneous diseases recently reclassified as ciliopathies, where causative genes encode ciliary or basal body proteins and the function and/or the structure of cilia, sensory organelles, are abnormal. One of the most intriguing JS genes is CEP290 as it has the widest phenotypic spectrum ranging from isolated blindness to in utero lethal Meckel-Grüber syndrome and mutations of this gene are responsible for 50% of JS with renal and ocular involvement (CORS). It is totally unknown how CEP290 and ciliary loss of function impair human cerebellar growth giving rise to the drastic vermis malformation characteristic of JS. Here we show that Shh dependent growth of human cerebellum, supported by the granule cells progenitors (CGP) proliferation, starts around 15 gw, that GCP possess a primary cilium and that CEP290 is localized at its base. We find that GCP proliferation and response to SHH is severely impaired in JS cases bearing mutations in genes including CEP290 or MKS3, as well as in a Jeune syndrome ciliopathy case and a Thanatophoric dysplasia case, a putative ciliopathy. Finally, we show that in JS cases, GCP proliferation and response to SHH are impaired not only in the vermis but also, and to the same extent, in the hemispheres. These results add a feature to the already described JS defects and rule out impaired GCP expansion as the origin of vermis hypo/aplasia. Our results show that Shh dependent GCP proliferation and consecutive cerebellar growth starts around 15 gw in the human cerebellum and is impaired in JS whole cerebellum. They also suggest that this could be a common feature to several ciliopathies.

S04.2
Ciliary control of feeding behavior and body weight

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Primary cilia have essential functions in regulating signaling pathways during mammalian development. In adults, cilia are also important as evidenced by the spectrum of phenotypes observed in mouse models and human patients with ciliary defects (ciliopathies); however, their roles in tissue physiology are poorly understood and the mechanisms underlying most ciliopathies remain enigmatic. We are addressing this issue using conditional tissue-specific cilia mouse mutants and show that disruption of cilia in the hypothalamus results in hyperphagia and obesity. A similar obesity phenotype was reported in mouse models of Bardet-Biedl Syndrome (BBS). BBS mutants have defects in receptor transport into the cilium and are thought to be unable to sense the anorexigenic peptide leptin. Here we assess the leptin signaling axis in cilia mutants and demonstrate they are sensitive to leptin prior to becoming obese, are leptin insensitive when obese, and then regain leptin sensitivity after caloric restriction to bring mutants back to wild type body weight. Thus leptin resistance is a secondary consequence of the obesity and not causal indicating that pathways other than the leptin-melanocortin axis are involved. Data indicate that one of these pathways could be the melanin concentrating hormone (MCH) axis. Previous data indicate that MCH receptor localizes in the cilium of control but not of BBS mutants. Further, we show that MCH binds MCHR1 in the cilium and that in the absence of the cilium, the MCH signaling pathway is altered. Pharmacological and genetic studies are being conducted to assess whether defects in MCH signaling are directly associated with the obesity phenotype in cilia mutants. This work will provide novel insights into how dysfunction of the cilium contributes to the onset of obesity and more generally the importance of the cilium in regulating normal neuronal activity and energy balance.

S04.3
Joubert syndrome

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Joubert syndrome is characterized by congenital ataxia, oculomotor apraxia and metal retardation and the pathognomonic Molar Tooth Sign on brain MRI (MTI). Joubert syndrome and related disorders (JSRD) refers to a multiorgan class of conditions displaying the MTI as well as frequent retinal blindness, nephronophthisis, hepatic fibrosis and polydactytly. JS and more broadly JSRD are caused by mutations in at least 10 different genes that are unified by the finding that the encoded proteins localize at or near the cellular primary cilium. Up until recently, neurons were not generally regarded to have primary cilia, but with better cilia visualization tools we now know that the most or all neurons in the developing and adult brain display primary cilia. Furthermore, mutations in these genes frequently result in altered structure of the primary cilium. Two competing models have emerged to explain the genotype-phenotype correlations: 1] These genes determine the structure of the primary cilium, alterations of which result in disease. 2] These genes modulate the efficiency of signaling of the many pathways linked to the primary cilium including Platelet derived growth factor, Sonic hedgehog and Wnt signaling.
The genes mutated in Joubert syndrome suggest that altered structure and/or function of the primary cilium underlie this complex phenotype, but many important questions remain: 1] What can the genes identified to date tell us about the basis of this class of disease? 2] What type of genetic modifiers can explain the widely variable expressivity in this disease? 3] What types of treatment options will emerge from the molecular characterization of this disease?

S05.1
Mechanisms and evolution in mammalian transcriptional regulation

***D. T. Odom**[1,2];*
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My laboratory investigates the complex relationships among tissue-specific transcription, genetic sequence, and evolution using high-throughput methodologies. We have demonstrated the remarkable divergence in tissue-specific transcriptional regulation that has occurred among mammals. We found that a set of liver-specific transcription factors whose function, amino acid sequence, and targeted binding motif are highly conserved throughout mammals changes both their potentially targeted genes and also their precise binding locations globally. An outstanding mechanistic question arose from the discovery of the rapid divergence of transcription factor binding: what directs these transcription factor binding changes? Is it genetic sequence, epigenetic state, organismal development, or some combination of these?
We showed that an aneuploid mouse line carrying a human chromosome could isolate genetic sequence as an independent variable in directing transcriptional regulation. Remarkably, mouse-encoded transcription factors bound the human chromosome in mouse liver almost precisely as do human-genome-encoded transcription factors in human liver. We found that the mouse nucleus could also accurately re-create the epigenetic landscape and even mRNA regulation across the entirely of this human chromosome. Thus, it is almost entirely genetic sequence variation that is responsible for interspecies differences in transcriptional regulation.
I will be discussing other current projects, including the evolution and conservation of CTCF binding events across multiple mammalian orders, and the manner that RNA polymerase III maintains its conserved transcriptome in the face of rapid remodeling of its transcribed tRNAs.

S05.2
Functional genomics in individuals: Understanding biology using intra-species comparisons.

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S05.3
Title to be announced

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S06.1
RNAi-based therapeutic strategies for inflammation and metabolic disease

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Infiltration of macrophages into adipose tissue in animal models of obesity increases expression and secretion of many factors which could potentially regulate lipogenesis and sequestration of fat in adipocytes. Recently our laboratory reported that human omental adipose tissue was more highly infiltrated with macrophages in obese, insulin resistant subjects, even when matched to equally obese human subjects that were insulin sensitive. We have initiated strategies to target macrophages with siRNA to suppress expression of genes that promote adipose dysfunction and metabolic disease. We have silenced TNF-α as well as other genes that promote inflammation using siRNA encapsulated within hollow shells of β, 1-3 D-glucan prepared from yeast cell walls (denoted GeRPs, for Glucan-encapsulated RNAi particles). Both oral and intraperitoneal administration routes have been tested in mice with GeRPs loaded with siRNA anchored between cationic (polyethyleneimine) and anionic (tRNA and siRNA) layers that also contain an amphipathic peptide. We have now developed simplified versions of these formulations with fewer components in order to enhance reproducibility of manufacture, promote siRNA release from GeRPs after phagocytosis and increase gene silencing potency. The newly developed GeRPs have achieved significant gene silencing in mice targeting Map4k4, a protein kinase that controls inflammation in marcrophages, as well as the cell surface proteins F4/80 CD40, CD80 and CD86 analyzed by FACS. The simplified GeRP system consists of glucan shells loaded in a step-wise procedure under various pH conditions with only an amphipathic peptide, Endo-porter, and siRNA. Current experiments are devoted to testing whether gene silencing in macrophages in vivo by these simplified GeRP formulations can alleviate insulin resistance in obese mouse models and in other animal models of inflammation.

S06.2
Gene therapy for inherited retinal dystrophies - from mouse to man

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S06.3
Gene therapy for genetic leukodystrophies

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S07.1
Causes and consequences of microRNA dysregulation in cancer

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During the past several years it has become clear that alterations in the expression of microRNA genes contribute to the pathogenesis of most, perhaps all, human malignancies. These alterations can be caused by a variety of mechanisms, including deletions, amplifications or mutations involving microRNA loci, by epigenetic silencing or by dysregulation of transcription factors targeting specific microRNAs. Since malignant cells show dependence on the dysregulated expression of microRNA genes, which in turn control or are controlled by dysregulation of multiple protein coding oncogenes or tumor suppressor genes, these small RNAs provide important opportunities for development of future microRNA based therapies.

S07.2
Evolution of the Cancer Genome

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All cancers carry somatically acquired changes in their genomes. Some, termed “driver” mutations, are causally implicated in cancer development. The remainder are “passengers”, and bear the imprints of mutational processes operative during cancer development. Following the advent of second generation sequencing technologies the provision of whole cancer genome sequences has become a reality. These sequences generate comprehensive catalogues of somatic mutations, including point mutations, rearrangements and copy number changes and provide insights into the evolutionary processes underlying the development of individual human cancers including the factors generating variation and the forces of selection. These insights will form the foundation of our understanding of cancer causation, prevention and treatment in the future.

S07.3
Roles of Chromatin ubiquitylation in DNA Double Strand Break Repair and Tumor Suppression

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Germline mutations to the Breast Cancer Early Onset 1 gene (BRCA1) confer a strong predisposition to breast and ovarian epithelial cancers. BRCA1 maintains genomic integrity by activating cell cycle checkpoints and error-free mechanisms of DNA double strand break (DSB) repair. Cancer causing mutations disrupt these activities primarily by impairing BRCA1 recognition and retention at DSBs. We have discovered that BRCA1 is targeted to DSBs via an interaction with the ubiquitin interacting protein, RAP80 (Sobhian et al. *Science* 2007). RAP80 provides a DNA damage recognition element by specifically binding lysine^{63}-linked ubiquitinated (K63-Ub) structures on chromatin adjacent to DSBs for a complex consisting of BRCA1 and several other proteins (Shao et al. *Genes & Development* 2009; Shao et al. *PNAS* 2009). These results imply that ubiquitin recognition is required for genome integrity and suppression of malignancy. Related to this hypothesis, new insights into the roles of this complex in DNA repair and tumor suppression will be presented. Recent findings from my laboratory have revealed that DSB associated ubiquitin also enables communication between DNA damage responses and transcriptional processes that occur on chromatin in cis to the site of damage (Shanbhag et al. *Cell* 2010). Using a novel assay that we have developed for single cell analysis, we have observed an ATM dependent process that silences transcription for multiple kilobases in cis to DSBs. New mechanistic insights into this ATM dependent DSB silencing pathway will be presented along with its implications to DSB repair, epigenetic inheritence, carcinogenesis, and viral latency.

S08.1
Mitochondrial quality control and neurodegeneration

***T. Langer**[1,2];*
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Dysfunction of mitochondria has severe cellular consequences and is linked to aging and neurodegeneration in human. Several surveillance mechanisms have evolved which prevent the accumulation of non-functional mitochondria and ensure cellular integrity. Whereas irreversibly damaged mitochondria can be selectively removed by autophagy, various intraorganellar proteases degrade non-native mitochondrial proteins and limit mitochondrial damage. These include hexameric m-AAA proteases, ATP-dependent proteases in the inner membrane of mitochondria, mutations of which are associated with hereditary spastic paraplegia and spinocerebellar ataxia (SCA28). m-AAA proteases are part of large supercomplexes with prohibitin scaffolds in the inner membrane. Recent experiments link the function of this protein complex to mitochondrial fusion and the processing of the dynamin-like GTPase OPA1, which is emerging as a central mechanism to monitor mitochondrial integrity. A dysfunction of mitochondria as well as the loss of prohibitins or m-AAA proteases induces the proteolytic breakdown of OPA1 isoforms by the OMA1 peptidase triggering mitochondrial fragmentation. Recent experiments on the role of this mitochondrial quality control mechanism for neuronal survival will be discussed.

S08.2
Insights into human neurodegenerative disease from Drosophila genetics

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The causes of amyotrophic lateral sclerosis (ALS) are poorly understood, although TDP-43 has been suggested to play a critical role. We found that ataxin 2, a polyglutamine (polyQ) protein mutated in spinocerebellar ataxia type 2 (SCA2), is a potent modifier of TDP-43 toxicity in yeast and Drosophila (Elden et al., 2010). Moreover, the proteins physically associate in an RNA-dependent manner, and ataxin 2 is abnormally localized in spinal cord neurons of ALS patients. Given these findings, we assessed the role of mutations in the gene encoding ataxin 2 (*ATXN2*) in ALS. Ataxin 2 bears a polyQ repeat which is normally 22 or 23 Qs, but becomes expanded to ≥34Qs in SCA2. We therefore considered that polyQ expansions greater than normal, but below the threshold for SCA2, may be associated with ALS. Analysis of 915 ALS patients revealed that intermediate-length polyQ expansions (27-33 Qs) in the *ATXN2* gene are significantly associated with ALS (4.7% of ALS cases vs 1.4% in controls).

The *ATXN2* gene presents additional interesting features because the polyQ expansions associated with SCA2 are typically pure CAG repeats. However, similar expansions that are interrupted with other codons in analysis to date present atypically with parkinsonism. Given the association of *ATXN2* with ALS, we determined the sequence of the *ATXN2* expansions in our ALS patients (Yu et al., 2011). These data indicate that expanded alleles associated with increased risk for ALS all bear at least one CAA interruption. These data suggest that the nature of the repeat sequence, as well as the length of the polyQ expansion, may play a role in neurological effects conferred by polyQ repeat expansions in the *ATXN2* gene.

Elden et al., 2010, Nature 466: 1069-1075.
Yu et al., 2011, PLoS One 6:e17951.

S08.3
When does neurodegeneration start in Huntington‘s disease: study of pre-symptomatic phase

***S. Tabrizi**;*
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Huntington’s disease is a neurodegenerative condition characterised by deterioration of motor and cognitive function as well as neuropsychiatric disturbance. The mean age of onset is 40 years, and progression is slow and inexorable, with death occurring typically 15-20 years later. It is inherited in an autosomal dominant manner. Currently there are no treatments available that modify disease progression. However, novel

therapeutic agents specifically targeting HD pathology are now on the horizon.
As Huntington's disease has a 100% sensitive and specific genetic test, it allows us to study at-risk subjects who carry the HD gene many years before symptom onset. The optimal point at which to introduce neuroprotective agents in the hope of delaying symptom onset and slowing the rate of disease progression is likely to be in the premanifest stage before the onset of rapid neuronal degeneration and emergence of clinical symptoms. Data from a conditional Huntington's disease mouse model suggests the potential reversibility of this neuronal dysfunction leading to full recovery when expression of the mutant gene is halted.
Studies such as TRACK-HD and PREDICT-HD have advanced our understanding of the clinical manifestations and underlying pathobiology of HD in the earliest phase of the neurodegenerative disease process, such that we are now better equipped to enter disease- modifying clinical trials and offer hope to the families affected by this devastating disease. In TRACK- HD, which is a large multi-national observational study, we are investigating a range of potential measures and biomarkers in premanifest HD gene carriers and early HD subjects which will have utility in clinical trials, and also help us understand the neurobiology of the premanifest and early phase of Huntington's disease.
In this talk I will summarise the state of the art in understanding the presymptomatic phase of neurodegeneration in Huntington's disease.

S09.1
Old men and selfish spermatogonia: how much do they contribute to the mutation burden?

A. O. M. Wilkie[1], *J. Lim*[1], *G. McVean*[2], *G. Turner*[3], *G. K. Jacobsen*[4], *E. Rajpert-DeMeyts*[5], *A. Goriely*[1];
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Gain-of-function mutations in 5 genes (RET, FGFR2, FGFR3, PTPN11 and HRAS) that cause congenital malformations exhibit the collective properties of very high apparent rates of germline base substitution, near-exclusive paternal origin, and increased average age of the father from whom the mutation has arisen (paternal age effect, PAE). Somatic mutations in these same genes have been described in various human cancers.
We have developed methods to quantify several of these mutations in human sperm and have also identified FGFR3 and HRAS mutations in spermatocytic seminoma, a rare type of testicular tumour. The combined evidence suggests that these mutations, although occurring rarely, provide a selective growth advantage to the mutant spermatogonial cell, resulting in clonal expansion over time, accounting for the PAE. To date, all examples of these "selfish" spermatogonial mutations locate within a single signalling pathway, the growth factor receptor-RAS pathway, which is a key determinant of spermatogonial stem cell proliferation and renewal. Our recent immunohistochemical analysis of normal testes shows features consistent with the occurrence of mutational microclones; a variety of patterns of antigen positivity are observed, consistent with different underlying driver mutations.
Regulation of cell turnover is important in many disease contexts, for example neurogenesis and neoplasia, so the consequences of mutations that hijack this process within the testis are potentially far reaching. Depending on the spectrum of average PAE mutations levels, they may contribute significantly to the 'dark matter' in human heritability, currently speculated to be explained by uncommon alleles of moderate effect. Hence this mechanism is likely to be important in the origins of common complex diseases such certain cancers and psychiatric disorders, as well as in congenital malformations.

S09.2
Telomeres, aging and stem cells

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S09.3
DNA damage and its implications for the aging epigenome

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Changes in chromatin structure are a conserved hallmark of aging and age-related diseases. The mechanisms driving these changes as well as their functional significance are heavily investigated. Over the past few years, it has become evident that DNA damage is a major contributor to a wide range of direct (genomic) and indirect (epigenomic) changes observed in aged nuclei, placing it at the crossroads of age-associated nuclear defects and functional decline. Recently, we were able to show that DNA damage associated epigenomic changes can be caused by the redistribution of the chromatin modifier SIRT1 from promoters and repetitive DNA to sites of DNA breaks, resulting in gene expression changes at ostensibly undamaged genomic loci. This SIRT1-associated gene deregulation correlated with a significant, albeit small fraction of age-related epigenetic changes. Preliminary data from our lab now implicate numerous other chromatin modifiers in DNA double-strand break (DSB) repair, suggesting a more general role for DNA damage-induced epigenomic reorganization in the aging process. Specifically, our data indicate that a repressive chromatin structure may be a critical and novel aspect of DSB repair, involving a significant nuclear reorganization of factors associated with age-related heterochromatin formation. Unlike DNA damage itself, damage-associated epigenetic changes are, at least theoretically, reversible and may, thus, provide a unique means to interfere with age-related nuclear reorganization and concomitant tissue dysfunction.

S10.1
Using custom arrays to improve the understanding of metabolic disorder

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Establishing the statistical significance of novel associations from genome-wide studies through replication as well as the fine-mapping of established loci to identify causal alleles are both crucial activities for understanding the genetics of complex traits in humans. As our understanding of human traits has increased over the past several years, new technologies are required to continue the accelerated pace of genetic discovery in performing both replication and fine mapping experiments. Each of these experiments requires hundreds of thousands of SNPs to be genotyped in thousands of samples and, until very recently, comprehensive application of these experiments has been cost prohibitive. To overcome this challenge, I describe the approach to the design, as well as the features, of a custom genotyping assay, the Metabochip, tailored to interrogate high priority genetic loci implicated in metabolic disorder and clinically important anthropometric measurements. After a brief characterization of the properties of the array, I will focus on insights gained from extensive replication studies for type-2 diabetes: namely, (1) how the array provides clues to the extent of polygenicity of this trait, and (2) how the depth of replication results can be used to extract new biological insight, using text-based mining approaches or protein-protein interaction networks. The Metabochip, and other similarly designed custom genotyping technologies like it, offer a practical and powerful experiment to advance the understanding of complex traits in humans.

S10.2
Ankylosing Spondylitis

M. A. Brown, *Wellcome Trust Case-Control Consortium 2, Australo-Anglo-American Spondyloarthritis Consortium (TASC), The Spondyloarthritis Research Consortium of Canada (SPARCC);*
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Ankylosing spondylitis (AS) is an inflammatory arthropathy affecting primarily the spine and pelvis, with a disease prevalence of 0.4% in white Europeans. Susceptibility to AS is known to be highly heritable (h2>90%), and the major gene for the condition, HLA-B27, has been known since 1972. However, only 1-5% of HLA-B27-carriers develop AS, and 10-20% of cases do not carry the gene, suggesting that other genes must be involved. Genetic studies of AS have made rapid progress over the past 5 years, with associations with novel genes

and genetic loci providing insights into the disease pathogenesis, stimulating much follow-up functional research and therapeutic development.
In order to identify further genetic variants predisposing to AS, a gwas was performed involving 1782 British and Australian cases fulfilling modified New York Criteria, and 5167 historical controls from the Wellcome Trust Case Control Consortium 2 (WTCCC2). The study was combined with existing results from the Australo-Anglo-American AS Consortium (TASC) consortium, and replication of the most significant findings performed in 2109 cases and 4410 controls from Australia, Britain, and the Canadian SPARCC consortium. As well as confirming known associations at *HLA*, *IL23R*, *ERAP1*, *KIF21B, 2p15* and *21q22,* we identified and subsequently replicated risk predisposing variants in *RUNX3*, *IL12B*, and *LTBR*, and found suggestive association at *ANTXR2*, *PTGER4*, *CARD9*, *TRADD* and *TBKBP1-TBX21*.
Additionally, we identified a single SNP within the MHC, rs4349859, which lies near the gene *MICA* and tags HLA-B27 with near perfect sensitivity (98%) and specificity (99%). Studies in cases and controls carrying different HLA-B27-subtypes, some AS-associated and some not, indicate that rs4349859 is not itself AS causative, and further reduce the likelihood that an *HLA-B*27*-linked gene is responsible for the association of B27 with AS. This SNP is far cheaper and easier to genotype than *HLA-B*27*-itself, and can be used as an alternative to *HLA-B*27*-typing, at least in European populations.
In most common diseases the proposed genetic model involves interaction between loci, but to date no convincing examples of such interaction have been demonstrated. We identified an interaction between *HLA-B*27* and variants within *ERAP1* in the WTCCC2 (p=0.02), TASC (p=0.014) and replication datasets (p=0.0019). Specifically, risk variants in *ERAP1* increased odds of disease in *HLA-B*27*-positive, but not *HLA-B*27*-negative, cases (combined $P=7.3x10^{-6}$). In contrast, SNPs at other AS-loci, including *IL23R, KIF21B, IL12B* and the intergenic regions 2p15 and 21q22, were AS associated in both *HLA-B*27*+ve and *HLA-B*27*-ve disease. This result indicates that *HLA-B*27*+ve and *HLA-B*27*-ve forms of disease have substantially different but overlapping aetiologies. Interestingly, in a separate WTCCC2 study, we have demonstrated that *ERAP1*is associated with psoriasis, and that the association is restricted to cases carrying the HLA Class I protein HLA-Cw6. The findings support mechanisms of association of *HLA-B*27* and *ERAP1* with AS that involve peptide presentation, and that ERAP1 contributes to disease risk through its action in trimming peptides prior to loading into nascent HLA class I molecules, rather than by cleaving pro-inflammatory cytokine receptors on the cell membrane.
Many further genes are likely to be identified over the coming years. The International Genetics of Ankylosing Spondylitis Consortium will perform what will likely be the definitive SNP genotyping study of AS, involving over 12,000 cases and 20,000 healthy controls genotyped for 200,000 markers targeting immunogenetically relevant genes using the 'Immunochip'. This study will bring new findings including new genes, fine-mapping of known genes, data about the value of genetic risk prediction in different world populations, and even pharmacogenetic data regarding TNF-antagonist response and intolerance.
These findings provide substantial material for the functional biology community to research, with the promise of early translation in both therapeutics and improved diagnostic tests.

S10.3
Genetic Analysis of Autoimmune Disease in the Wellcome Trust Case-Control Consortium 2

***C. Spencer**, on behalf of the WTCCC2;*
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I will discuss genome-wide association studies of Psoriasis, Ankylosing Spondylitis and Multiple Sclerosis performed as part of the Wellcome Trust Case-Control Consortium 2 (WTCCC2)

S11.1
Dog: how comparative genetics inform human diseases

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S11.2
Drosophila: Parkinson's disease

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Neurodegenerative diseases present an increasing challenge to biomedical science. Our understanding of the cause and nature of diseases is greatly informed by study of models organisms that recapitulate features of the disease. One model system that has proven surprisingly tractable for these diseases is the fruit fly, *Drosophila melanogaster*. In my lab we are principally focussing on understanding the function of genes linked to Parkinson's disease (PD). PD is the second most prevalent neurodegenerative disorder principally affecting the dopaminergic neurons of the substantia nigra. The pathogenic mechanisms are unknown and there is currently no cure or disease-modifying therapies. Genetic studies have begun to identify single-gene mutations responsible for rare heritable forms of PD and define genetic risk factors contributing to disease prevalence in sporadic cases. These findings provide an opportunity to gain insight into the molecular mechanisms of this disorder through the creation and analysis of appropriate genetic models. Analysis of a number of Drosophila models of PD has revealed some profound and sometimes surprising insights into PD pathogenesis. Moreover, these models can be used to investigate potential therapeutic strategies that may be effective in vivo, and tests have highlighted the efficacy of a number of neuroprotective compounds. I will discuss the methodologies employed in developing the various Drosophila models, and the recent advances that these models in particular have contributed to our understanding of the mechanisms that underlie PD pathogenesis and possible treatment strategies.

S11.3
Modeling Parkinson's disease in zebrafish

***O. Bandmann**;*
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Parkinson's Disease (PD) is the second most common neurodegenerative disease after Alzheimer's Disease. The pathological hallmark of PD is loss of dopaminergic neurons in the substantia nigra (SN). Currently, only symptomatic treatment is available. The identification of monogenically inherited subtypes of PD has provided us with crucial new insight into the pathogenesis of this disorder, but not led to better therapy as such. Mitochondrial dysfunction is of crucial importance in the pathogenesis of both sporadic and familial PD.
Zebrafish (*Danio rerio*) are emerging as a new animal model for human disease. They are vertebrates and therefore more closely related to humans than other model systems such as *c. elegans* or *drosophila*. There is very high sequence homology of many PD genes between humans and zebrafish. The embryos develop externally and are transparent. The dopaminergic nervous system in zebrafish is well characterized.
We will use PD as an example how zebrafish can be used to study human disease. Knockdown of *parkin* (an autosomal recessively inherited PD gene, leading to the disease due to loss of function) leads to loss of dopaminergic neurons and mitochondrial dysfunction with specific lowering of complex I activity, mirroring both the biochemical and pathological abnormalities observed in human PD patients with *parkin* mutations. A STOP mutation in *PINK1*, a further autosomal recessively inherited, loss of function PD gene also results in mitochondrial dysfunction and loss of dopaminergic neurons. In addition, *PINK1* mutant zebrafish larvae display a behavioural phenotype compatible with the abnormalities observed in human patients.

S12.1
PGD - 21 years on

***J. Harper**;*
UCL Centre for PG & D, EGA Institute for Womens Health, University College London, London, United Kingdom.

Preimplantation genetic diagnosis (PGD) was developed in 1989 as an alternative to prenatal diagnosis to help couples at risk of transmitting a genetic disease to have a healthy family. To achieve this patients go through in vitro fertilisation (IVF) procedures. Cells are removed from the embryo at one of three stages; polar bodies from the oocyte/

zygote, blastomeres from cleavage stages or trophectoderm from blastocysts. During the last 21 years, the majority of cycles have been performed using cleavage stage biopsy. The diagnosis has mainly been performed using the polymerase chain reaction (PCR) for monogenic disorders and fluorescent in situ hybridisation (FISH) for chromosome analysis.

This technology has also been used as an adjunct to IVF to help choose the 'best' embryo for transfer by testing for as many chromosomes as possible; preimplantation genetic screening (PGS). PGS has been a controversial procedure as thousands of cycles have been performed, but eleven randomised controlled trials (RCT) have shown that it does not increase IVF delivery rates for certain indications.

The European Society of Human Reproduction and Embryology (ESHRE) set up an international PGD consortium in 1997 to monitor PGD and collect data on PGD cycles worldwide. We have collected data on over 33,000 cycles of PGD/PGS, produced four guidelines on PGD/PGS and have five working groups.

Recent advances in the field of PGD/PGS have been the exploration of polar body biopsy (for those countries where embryo biopsy is not allowed) and blastocyst biopsy. Improvements in embryo freezing by using vitrification have resulted in an increase in pregnancies from biopsied embryos. The use of array-comparative genomic hybridisation (a-CGH) or single nucleotide polymorphism arrays (SNP-arrays) is rapidly entering the PGD/PGS arena. Many groups are performing blastocyst biopsy, vitrifying the embryos, and using array technology to perform the diagnosis. The embryos are replaced in a future cycle. For PGS, the new technology needs to be shown to improve delivery rates by performing RCTs.

Controversially, PGD has been used for social sexing, the diagnosis of late onset disorders and HLA matching. Treatment of these indications has caused many ethical discussions and the use of whole genome scanning will bring more debate.

S12.2
Use of aCGH in Prenatal Diagnosis

***A. L. Beaudet**;*

Department of Molecular and Human Genetics, Houston, TX, United States.

Array comparative genomic hybridization (aCGH) is currently being offered as a rapid and reliable method of prenatal testing for the detection of structural and numerical chromosome abnormalities. aCGH can survey the entire genome for submicroscopic deletions and duplications, aneuploidy and other unbalanced chromosomal abnormalities and is being increasingly applied to prenatal diagnosis, where it is already making significant impact. Inclusive of our reported experience with 300 prenatal cases (PMID 19012303), we now have aCGH data on 841 clinical prenatal samples, 545 (65%) of which were direct amniotic fluid or direct CVS specimens, allowing for a turnaround time far superior to that of cultured cells. Copy number changes were detected in 166 (20%) of the total 841 cases. Of these, 94 (11%) were CNVs that were interpreted as likely benign either because they were inherited from a phenotypically normal parent (90) or they were *de novo* (4) but had been previously observed in our aCGH database of over 26,000 clinical cases and phenotypically normal individuals. Of the 63/841 (7.5%) cases in which clinically significant genomic imbalances were detected, 23/841 total cases (2.7%) or 23/63 abnormal cases (36.5%) would not have been detected by conventional G-banded prenatal chromosome studies alone. These data demonstrate the superior diagnostic power of aCGH compared to conventional karyotype analysis, with a relatively small number of findings of uncertain significance (1% or less), most of which can be resolved by analyzing parents or by using data from ever-growing experience with diagnostic aCGH. It is noteworthy that we have now analyzed more than 400 prenatal samples on high-resolution oligo arrays and the frequency of findings of uncertain significance has remained at approximately 1%. Furthermore, the increasing availability and decreasing cost of prenatal aCGH will likely lead to its universal acceptance as a first-line prenatal diagnostic test for fetal chromosomal abnormalities in the near future. Furthermore, we have successfully optimized aCGH technology to achieve detection of copy-number imbalances of 1 Mb from single cells; with improving technology for fetal cell or fetal DNA enrichment, this may open up new avenues for non-invasive prenatal diagnosis.

S12.3
Non-invasive prenatal diagnosis using circulating fetal DNA: state of the art

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The discovery of the presence of cell-free fetal DNA in maternal plasma in 1997 has opened up new possibilities for non-invasive prenatal diagnosis. Early work had focused on the detection of paternally-inherited targets in maternal plasma, e.g. Y chromosomal markers. Recently, the development of technologies for analyzing single DNA molecules, e.g. digital PCR and next-generation sequencing, has greatly enhanced our ability to exploit the diagnostic capabilities of circulating nucleic acids. Hence, using next-generation sequencing, fetal trisomy 21 can be detected with high sensitivity and specificity from maternal plasma (Chiu et al. BMJ 2011; 342: c7401). Furthermore, it has been demonstrated in a proof-of-concept study that the entire fetal genome can be scanned non-invasively by maternal plasma DNA sequencing (Lo et al. Sci Transl Med 2010; 2: 61ra91). It is envisioned that plasma DNA analysis will play an increasingly important in prenatal diagnosis and monitoring.

S13.1
Genomic imprinting: an epigenetic gene regulatory model

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Genomic imprinting is an epigenetic process leading to parental-specific expression of 1-2% of mammalian genes that offers one of the best model systems for a molecular analysis of epigenetic regulation in development and disease. In the twenty years since the first imprinted gene was identified, this model has had a significant impact on decoding epigenetic information in mammals. So far it has led to the discovery of long-range *cis*-acting control elements whose epigenetic state regulates small clusters of genes, of unusual macro ncRNAs that directly repress genes *in cis* and critically, it has demonstrated that one biological role of DNA methylation is to allow expression of genes normally repressed by default. Here we describe our progress in understanding how imprinted protein-coding genes are silenced, in particular, focussing on the role of macro (or long) ncRNAs that have broad relevance as a potential new layer of regulatory information in the mammalian genome. The *Airn* macro ncRNA silences the *Igf2r* protein-coding gene on the paternally inherited copy of mouse chromosome 17. We have previously established an *in vitro* model in differentiating ES cells that emulates the developmental onset of imprinted expression of *Igf2r* and *Airn* (Latos et al., 2009). The ES cell model was used to determine the functional length of *Airn.* Several ES lines carrying different *Airn* truncations were generated and tested for their ability to direct imprinted *Igf2r* expression and to change the physical characteristics of the *Airn* ncRNA itself. The data show the *Airn* ncRNA sequence and structure do not play a role in *Igf2r* repression. Since the *Airn* transcript overlaps the *Igf2r* promoter, we next tested if transcriptional overlap was sufficient to repress *Igf2r* by moving the *Airn* promoter directly in front of the *Igf2r* promoter. Together, the results do not support a functional role for the *Airn* ncRNA but, instead, indicate that transcriptional overlap by the *Airn* ncRNA is sufficient to repress *Igf2r* in cis.

S13.2
How epigenetics disconnects the ability to learn with increasing age

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Aging is a major issue in modern societies since it is the main risk factor for dementia, especially Alzheimer's disease. It has been shown across various species including humans, monkeys and rodents that age-associated memory impairment (AAMI) correlates with deregulated gene expression in certain brain regions such as the hippocampus. During the last years it became increasingly evident that genome-environment interactions have a significant impact on cognitive functions and that these GxE interactions are mediated by epigenetic mechanisms like DNA methylation and post-translational

histone modifications.
Recent findings in our laboratory raise the possibility that acetylation on Lysine 12 of Histone 4 (H4K12ac) is an important regulator in dynamic transcription of learning associated genes. H4K12ac was the only of several investigated acetylation sites on H4 and H3 that did not respond to a learning stimulus in the fear-conditioning paradigm in a mouse model of early aging (16-month-old mice). The finding that H4K12ac seems to be especially important for transcriptional elongation links this modification to the almost complete loss of transcriptome plasticity in 16-month-old mice. H4K12 acetylation as well as memory performance could be reinstated by intrahippocampal injection of an HDAC inhibitor in old individuals.
Furthermore, we employed 24-month-old mice that represent a model for advanced aging. The 24-month old mice showed significant impairment in the novel object recognition (NOR) learning paradigm. By employing a combination of ChIP-seq and DNA microarray we were able to define the epigenetic landscape of H4K12 acetylation and the hippocampal transcriptome of these mice. We observed elevated genome-wide levels of H4K12ac, which corresponded to a high degree of differential gene expression in the aged hippocampus. In summary, histone acetylation-mediated GxE interactions seem to be an essential regulator of stimulus-driven transcription and a hallmark of age-associated deficits and diseases.

S13.3
Towards epigenome-wide association studies (EWAS)

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What determines a phenotype is one of the fundamental questions in biology and medicine. In addition to genetic factors, epigenetic factors such as DNA methylation have been shown to play important roles. To understand the rules governing DNA methylation and their functional consequences requires genome-wide analysis of methylome dynamics. I will present our efforts using array- and sequencing-based platforms for high-throughput methylome analysis, discuss some of the lessons learnt and give an outlook on how this approach may be used for epigenome-wide association studies (EWAS) to analyse and better understand phenotypic plasticity in health and disease.
For further details, please see:
http://www.ucl.ac.uk/cancer/research-groups/medical-genomics

S14.1
Genomic advances in early-onset myocardial infarction

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S14.2
CCM protein interactions, double KO mouse models

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S14.3
Genomics of blood pressure and hypertension

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The presentation will describe the initial difficulties experienced in early genome wide association studies of blood pressure and hypertension as well as strategic developments including large meta-analyses and sampling from the extremes of blood pressure distribution. Published and in press successes, which include more than 20 SNPs/loci associated with blood pressure and hypertension, will be described together with the future directions such as exome sequencing as well as clever functional studies leading to clinically useful applications.

S15.1
Jewish population structure

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I shall give a brief overview of the genetic studies that have addressed Jewish origins including studies focusing on uniparentally and biparentally inherited markers but will focus on genome-wide patterns of variation across the vast geographic span of Jewish Diaspora communities and their respective neighbours.
We have used Illumina 6X0 bead arrays to genotype individuals from 20 Jewish Diaspora communities, including in addition to the European communities those from Northern Africa, India and Central Asia, and compare these patterns of genome-wide diversity within the context of the genetic landscape of the Old World. We have also improved the sampling of the host populations and those from the historically suggested region of Jewish origins in the Middle East.
Principal component and structure-like analyses show that most Jewish samples shear genetic ancestry signals and form a sub-cluster that overlies with some Levantine populations but not the paired Diaspora host populations. Nevertheless, in line with the history of the different Jewish communities, different levels of admixture with the host populations can be identified. In the extreme, Ethiopian Jews (Beta Israel) and Indian Jews (Bene Israel and Cochini) cluster with neighbouring autochthonous populations in Ethiopia and western India, respectively, despite a clear paternal link between the Bene Israel and the Levant.
Overall these results point to the genetic origins of most Jewish Diaspora communities in the Levant and exemplify how genetic information may be utilized to address questions of demographic history where historical evidence is weaker or inexistent.

S15.2
A fine-scale map of recombination rates and hotpots in the chimpanzee reveals conservation and turnover in factors localising cross-overs.

***O. Venn**[1], A. Fledel-Alon[2], A. Auton[1], L. Segurel[2], S. Pfeifer[3], E. Leffer[2], R. Hernandez[4], C. Melton[2], R. Bowden[1,3], Z. Iqbal[1], I. Turner[3], J. Maller[3], G. Lunter[1], J. Broxholme[1], P. Humburg[1], S. Myers[1,3], P. Donnelly[*1,3], M. Przeworski[*2,5], G. McVean[*1,3];*
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S15.3
Genome diversity in Asia

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Asia harbors substantial cultural and linguistic diversity, but the geographic structure of genetic variation across the continent remains enigmatic. Here we report a large-scale survey of autosomal variation from a broad geographic sample of Asian human populations. Our results show that genetic ancestry is strongly correlated with linguistic affiliations as well as geography. Most populations show relatedness within ethnic/linguistic groups, despite prevalent gene flow among populations. More than 90% of East Asian (EA) haplotypes could be found in either Southeast Asian (SEA) or Central-South Asian (CSA) populations and show clinal structure with haplotype diversity decreasing from south to north. Furthermore, 50% of EA haplotypes were found in SEA only and 5% were found in CSA only, indicating that SEA was a major geographic source of EA populations.

S16.1
Research Governance - So…Last Century?

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Since the advent of the Human Genome Project, we have seen significant changes in the way that research is carried out. Samples and data are now shared widely on a global level by researchers in international consortia and biobanking networks. The problem is that our research governance systems are nationally based, following national legal requirements and guidelines, and relying on the expertise of research ethics committees for oversight. In this paper I will argue that our current national research governance systems are inadequate for the task of international data sharing and that radical new approaches are needed to bring our research governance systems into the 21st century.

S16.2
Feedback of individual genetic results to research participants: in favor of a qualified disclosure policy

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In recent years, a debate evolved regarding the question whether researchers have a duty to return individual genetic research results to research participants. This unsettled debate has rapidly gained in urgency in view of the emergence of biobanks and the advances in next-generation sequencing technology, which has the potential to generate unequalled amounts of genetic data. This implies that the generation of many known and unknown genetic variants in individual participants of genetics/genomics research as intentionally or collaterally obtained by-products is unavoidable.

In both the scientific debate and in international guidelines, the extreme positions of full disclosure and no disclosure whatsoever are seldom defended. A duty to warn when this may save the life of a research participant is apparently recognized widely. As no disclosure is unethical because it fails to adhere to the so-called rule of rescue and full disclosure nonsensical (at best) as it could imply disclosure of all raw sequencing data, any disclosure policy in between means we have to consider how and by whom a selection should be made about which results are eligible for disclosure.

In this presentation, a qualified disclosure policy is proposed. This policy contains a standard default package, possibly supplemented with three additional packages. Whereas the default package, containing life-saving information of immediate clinical utility, should be offered routinely and mandatory to all research participants, offering (one of) the three additional packages is context-specific. Such a qualified disclosure policy in our opinion best balances the potential benefits of disclosure with the potential risks for research participants and the harms of unduly hindering biomedical research.

S16.3
Ethics, genetics and the family

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ES1.1
Mitochondrial diseases

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The first pathogenic mutations of mitochondrial DNA (mtDNA) were identified over 20 years ago, paving the way for two decades of clinical and molecular research, and opening up the new field of mitochondrial medicine. Pathogenic mutations of mtDNA have been found in > 1 in 200 of the general population, and cause mitochondrial disease in > 1 in 5000. The spectrum of mitochondrial disorders continues to increase, both at the molecular and clinical level, presenting new challenges for diagnosis and treatment.

This presentation will provide the basic biochemical and molecular knowledge required to understand mitochondrial disorders in a clinical context, describe novel phenotypes that present in adult life, discuss the complex interplay between the nuclear genome and the mitochondrial genome in human disease, and describe new treatments under development for these disorders.

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2. Yu-Wai-Man P, Griffiths PG, Gorman GS, Lourenco CM, Wright AF, Auer-Grumbach M, Toscano A,□.Chinnery PF. (2010) Multi-system neurological disease is common in patients with OPA1 mutations. Brain 133:771-786
3. Yu-Wai-Man P, Sitarz KS, Samuels DC, Griffiths PG, Reeve AK, Bindoff LA, Horvath R, Chinnery PF (2010) OPA1 mutations cause cytochrome c oxidase deficiency due to loss of wild-type mtDNA molecules. Hum Mol Genet 19:3043-3052
4. Samuels DC, Wonnapinij P, Cree LM, Chinnery PF (2010) Reassessing evidence for a postnatal mitochondrial genetic bottleneck. Nat Genet 42:471-472
5. Craven L, Tuppen HA, Greggains GD, Harbottle SJ, Murphy JL, Cree LM, Murdoch AP, Chinnery PF, Taylor RW, Lightowlers RN, Herbert M, Turnbull DM (2010) Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease. Nature 465:82-85

ES1.2
Neonatal screening for inherited diseases

***U. von Döbeln**[1,2];*
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Mass screening of newborn infants by analysis of blood samples dried on filter paper (DBS) is performed all over the world. The aim is to find infants with treatable inborn diseases in order to improve the long term outcome by early institution of treatment.

Neonatal screening started with the autosomal recessive disorder phenylketonuria (PKU) in the early sixties by the pioneering work of Robert Guthrie. He developed a bacterial assay on DBS that could detect infants with this serious disorder before it had given symptoms. Patients with PKU born before the start of screening are mentally retarded while those treated since the neonatal period become completely normal.

New disorders (eg galactosemia, congenital hypothyroidism, congenital adrenal hyperplasia, cystic fibrosis) have been added one by one when analytical methods for the screening of them have been developed. Screening for congenital hypothyroidism is the most widely spread over the world. This disorder has a fairly high incidence (1:3000) and is easy to treat at a low cost with one pill of thyroxin a day.

In the early nineties a new era for neonatal screening started with the introduction of LC-MS-MS. With this equipment the concentration of more than a hundred metabolites can be quantified simultaneously in a 3 mm diameter punch of dried blood on filter paper at an analysis time of 2 min per sample. New technologies for DNA analyses will certainly also contribute to the development of neonatal mass screening.

LC-MS-MS has meant a technological break through but has also led to ethical and economical dilemmas with respect to choice of disorders to include in the screening panel.

This is well illustrated by the differences between countries. In the USA the panel comprises more than 54 disorders whilst at the other extreme Great Britain is screening for four.

ES2.1
Lynch syndrome (HNPCC)

***E. Mangold**;*
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Hereditary non-polyposis colon cancer (HNPCC), also called Lynch syndrome, is an autosomal-dominant predisposition to colon cancer and other malignancies (e.g., endometrium, urinary tract, small bowel, biliary tract, ovary, stomach cancer). Lynch syndrome patients and their first degree relatives are recommended lifelong surveillance for colorectal cancer and other Lynch syndrome malignancies starting in their 20s and 30s. The diagnosis can be made based on clinical data and family history (Amsterdam criteria) and/or when a causative germ line mutation has been detected. To date, private mutations in mismatch-repair genes MSH2, MLH1, MSH6, PMS2 or deletions of EPCAM leading to epigenetic silencing of MSH2, are known as molecular causes of Lynch syndrome. The identification of a causative mutation is important, as it allows predictive testing in healthy family members.

Not all Lynch syndrome patients meet the Amsterdam criteria for Lynch syndrome. The diagnosis of HNPCC should also be considered when the patient's individual history and family history meet one or more of the less stringent criteria (revised Bethesda guidelines). Among these patients an examination of tumor tissue for Lynch syndrome characteristics is able to detect those, who have a high probability for Lynch syndrome and therefore should undergo surveillance and mutation screening. A high microsatellite instability and loss of DNA mismatch repair protein expression are characteristic for Lynch syndrome tumors. However, a loss of MLH1 and PMS2 expression is also observed in a considerable portion of sporadic cancers.

This educational will focus on diagnostic approaches in patients diagnosed with or suspected of Lynch syndrome and point out possible pitfalls.

ES2.2
BRCA1 and *BRCA2* genes cancer susceptibility: from bench to bedside

***D. Stoppa-Lyonnet**[1,2];*
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The chromosomal mapping of the *BRCA1* gene by Mary-Claire King twenty years ago has opened the field of breast cancer genetics, and paved the way to the personalized management of women at-risk because of their breast cancer family history.

The first application of the identification of the *BRCA1* and *BRCA2* genes was indeed genetic testing. Yet, 20 years latter, *BRCA1/2* genetic testing remains complex because of the large size of the coding sequences, of the broad allelic heterogeneity - disease-predisposing mutations being different from a family to another in most cases -, and of the vast number of rare variants with unknown significance (VUS). The international ENIGMA consortium (Evidence based Network for the Interpretation of Germline Mutant Alleles) has been recently created to classify these VUS by using multiple approaches including family cosegregation data.

Tumour risk estimate is also a major concern. Numerous factors, both genetic and non-genetic, are influencing risks. Their identification, still ongoing, is of the utmost importance for the individualised management of at-risk women, and for opening new ways of prevention and treatment. Two international consortia, now associated, play a major role on their identification namely the IBCCS (International *BRCA1/2* Carrier Cohort Study) and CIMBA (Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2*) consortia, for non-genetic and genetic factors respectively.

The discovery of the involvement of the BRCA1 and BRCA2 proteins in the Double-Strand Break DNA repair pathway has lead to specific clinical trials testing molecules increasing the tumour genomic instability such as alkylating agents and Base Excision Repair inhibitors (PARPi). Their first results are promising and, importantly, could also have an impact on non-*BRCA1/2* related breast tumours, some of them showing DSB repair defects as well.

Thus, in this particular field, may be more than in any other genetic medicine areas, it is fascinating to see how much, in so few years, the distance between bench and bedside has shortened, at-risk women and researchers now walking next to each other.

ES3.1
eQTL

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No abstract received as per date of production. Please see www.eshg.org/eshg2011 for possible updates.

ES3.2
Design, analysis and interpretation of genome-wide association studies

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Genome-wide associations studies (GWAS) have revolutionised the field of human genetics and have directly led to the identification of over a thousand loci associated with complex traits and disease. In this presentation I will begin by outlining the rationale behind genome-wide association studies and the technological advances that were necessary to make them a reality. I will go on to discuss how to design a successful GWAS and outline some of the steps that should be undertaken to reduce false-positive and false-negative findings. I will then review GWAS replication studies and meta-analysis. Throughout the presentation I will draw on examples from the GWAS literature. I will finish by summarising the major lessons learnt from GWAS and discussing the 'next-generation' of genome-wide association studies.

ES4.1
Genetics of fear, anxiety and affective disorders

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No abstract received as per date of production. Please see www.eshg.org/eshg2011 for possible updates.

ES4.2
Autism

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No abstract received as per date of production. Please see www.eshg.org/eshg2011 for possible updates.

ES5.1
Targeted rapid aneuploidy detection in prenatal diagnosis

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No abstract received as per date of production. Please see www.eshg.org/eshg2011 for possible updates.

ES5.2
Cutaneous mosaicism 2011: understanding the patterns and mechanisms

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All nevi reflect mosaicism. Such skin lesions show a remarkable divergency of archetypical patterns. Some unusual types are the "Ruggieri pattern"of pigmentary disturbances, and the hypopigmented macules of Pallister-Killian syndrome. - Epigenetic cutaneous mosaicism can today explain the hereditary patterns of lyonization as well as hereditary autosomal pigmentary disturbances arranged along Blaschko's lines. - Genomic mosaicism: Such mosaics can usually not be transmitted to the next generation. A new possible example of a lethal mutation surviving by mosaicism is papular nevus spilus syndrome that is characterized by ipsilateral neurologic defects, especially in the form of segmental hyperhidrosis. Phylloid hypomelanosis is a novel neurocutaneous phenotype that reflects mosaic trisomy 13q. - The concept of type 2 segmental manifestation of autosomal dominant skin disorders, reflecting early LOH, has been confirmed by molecular analysis in six different skin disorders including NF1, Hailey-Hailey disease, Darier disease and rhodoid nevus syndrome ("capillary malformation-arteriovenous malformation"). - Conversely, in autosomal recessive epidermolysis bullosa, postzygotic loss of homozygosity may cause revertant mosaicism in the form of mosaic patches of healthy skin. Similarly, ichthyosis variegata (ichthyosis in confetti) reflects thousands of revertant clones involving the *KRT10* locus. - The concept of didymosis (twin spotting) explains the coexistence of two different mosaic patches such as capillary didymosis, cutis tricolor, or phacomatosis spilorosea. - Superimposed segmental manifestation of polygenic skin disorders: Clinical examples now include psoriasis, atopic eczema, lichen planus, systemic lupus erythematosus, dermatomyositis, pemphigus vulgaris, vitiligo, graft-versus-host disease, granuloma annulare, drug eruption, and multiple melanocytic nevi. This new genetic concept offers an explanation why in polygenic skin disorders "mixed" cases of both segmental and nonsegmental involvement may occur; why the segmental manifestation is rather severe, tends to occur early in life and is rather difficult to treat; and why family members may show the same disorder in a nonsegmental form.

ES6.1
Marfan syndrome: evolving nosology, evolving pathophysiology and evolving treatment with improved survival

***C. Boileau**[1,2,3];*
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Marfan syndrome (MFS), the prototypical heritable connective tissue disorder, has been known for its pleiotropy since Victor McKusick's founding monograph in 1955. Through the years, clinical pleiotropy has led to an evolving nosology (Berlin, 1986: Gent I, 1996; and Ghent II, 2010) that illustrates the difficulty of the diagnosis of MFS. Twenty years ago the syndrome was found to be due essentially to mutations within the *FBN1* gene that encodes fibrillin 1, the major component of 10 nm diameter microfibrils, matrix structures found as isolated aggregates or closely associated with elastin. Careful clinical investigations and the identification of molecular defects located in new genes (*TGFBR2* and *TGFBR1*) have lead to the recognition of overlapping syndromic entities: Marfan syndrome type 2 and the Loeys-Dietz syndrome. Conversely, *FBN1* gene mutations have been identified in familial diseases affecting mainly one organ system (isolated ectopia lentis, familial thoracic aortic aneurysm and dissection, Marfanoid habitus), thus showing that they are allelic to MFS. This molecular complexity of human diseases has benefited the understanding of pathogenic mechanisms underlying MFS since it opened avenues of research not only in the field of connective tissue remodelling, but also in alteration of TGF β signalling. Historically, MFS was thought to result from structural weakness of connective tissue. This was confirmed with the indentification of mutations (either missense or nonsense) in the *FBN1* gene associated with a dominant negative or haploinsuffisiency effect, respectively. Mutations could reasonably well explain the altered mechanical properties of the zonules (ocular ligaments containing high levels of isolated microfibrillar aggregates) and the aortic media (containing elastin lamellae closely bordered with microfibrils) in MFS. However, no clear genotype/phenotype correlation could be established between *FBN1* mutations and various forms of MFS but for the identification of an association between mutations in exons 24-32 and the neonatal MFS as well as the tendency to a more severe phenotype in adults. Further insight into pathophysiology has been gained through the study first of the transgenic *Fbn1* $^{mg\Delta}$ and subsequently the KI *Fbn1* ^{C1039G} mice strains, the latter providing a model more analogous to the situation in affected humans. The mice strains have led to the identification of increased TGF β signalling within disease tissues. This finding is in agreement with an additional role for fibrillin 1: matrix sequestration of inactive TGF β and regulation of TGF β activation by targeting and concentrating TGF β at specific locations through its interaction with latent TGF-β-binding proteins (LTBPs). The identification of altered TGF β signalling has led to several clinical trials worldwide to test the efficacy of Losartan on blunting the overstimulation of the signalling and thus on the aortic disease. The scientific challenge is now the identification of genetic modifiers that could lead to the identification of prognostic factors and new therapeutic targets.

ES6.2
The Ehlers-Danlos syndrome: a disorder with many faces

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Centre for Medical Genetics, Ghent University Hospital, Ghent, Belgium.

The Ehlers-Danlos Syndromes (EDS) comprise a heterogeneous group of diseases which are characterized by fragility of the soft connective tissues and widespread manifestations in skin, ligaments and joints, blood vessels and internal organs. The clinical spectrum varies from mild skin and joint hyperlaxity to severe physical disability and life-threatening vascular complications. Current classification (Villefranche Nosology) recognizes 6 genetic subtypes, most of which are linked to mutations in one of the genes encoding fibrillar collagen proteins or enzymes involved in post-translational modification of these proteins. Mutations in type V and type III collagen cause classic or vascular EDS respectively while mutations involving the synthesis or processing of type I collagen are involved in the kyphoscoliosis, arthrochalasis and dermatosparaxis type of EDS. Establishing the correct EDS subtype has important implications for genetic counselling and management and is supported by specific biochemical and molecular investigations. Over the last years, several new EDS variants have been characterized which call for a refinement of the Villefranche classification. Moreover, the study of these diseases has brought new insights into the molecular pathogenesis of EDS by implicating genetic defects in the biosynthesis of other extracellular matrix molecules, such as proteoglycans and tenascin, or genetic defects in molecules involved in intracellular trafficking, secretion and assembly of extracellular matrix proteins.

No causal therapy is available for EDS, but a series of "preventive" guidelines are applicable to all forms of EDS. Key-aspects of management include cardiovascular work-up, physiotherapy, pain management and psychological support. Recently, a clinical trial has shown promising results for a betablocker, Celiprolol, in the prevention of vascular catastrophies in patients with vascular EDS.

ES7.1
Noonan Syndrome

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ES7.2
Neurofibromatosis

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RAS proteins play key roles in normal cell growth, malignant transformation, learning and memory. Somatic mutations in *RAS* genes and several of their upstream and downstream molecules play an important role in different human malignancies.

Neurofibromatosis type 1 is caused by heterozygous mutations in the *NF1* tumor suppressor gene coding for neurofibromin. Neurofibromin functions as a GTP-ase activating protein towards RAS and neurofibromatosis type 1 was the first human inherited disorder to be associated with an increased signalling through the RAS-Mitogen Activated Protein Kinase (MAPK) pathway.

Most features of NF1 are the result of tissue specific inactivation of the normal *NF1* allele. "Second hits" in the *NF1* gene have been observed in NF1 related tumors such as neurofibromas, gastrointestinal stromal tumors, pheochromocytomas, brain tumors, and glomus tumors of the digits. "Second hits" were also seen in melanocytes from café-au-lait spots, and pseudarthrosis tissue. In addition patients with NF1 have an increased risk of malignant peripheral nerve sheath tumors.

These "second hits" in *NF1* result in a deficiency of RAS signalling down regulation with subsequent overactivity of the RAS pathway. Cognitive difficulties in NF1 patients and *Nf1* heterozygous mice have been attributed to haploinsufficiency for *NF1(Nf1)*. Pharmacological down regulation of RAS activity is capable of correcting learning and memory deficits in adult mice. Clinical trials in humans with NF1 trying to pharmacologically correct the learning disabilities are running at this moment.

Legius syndrome is a more recently described entity resembling neurofibromatosis type 1. It is caused by heterozygous mutations in *SPRED1*, a negative regulator of RAF activation by RAS-GTP. Legius syndrome patients show the same multiple café-au-lait spots as patients with NF1 but without Lisch nodules, neurofibromas, optic pathway gliomas and NF1 related bone abnormalities. Melanocytes from café-au-lait spots in Legius syndrome patients show a "second hit" in the *SPRED1* gene. Legius syndrome is associated with mild learning problems and the *Spred1* knockout mouse model shows similar learning and memory deficits as the *Nf1* mouse model.

These findings point to important roles for the evolutionary conserved RAS-MAPK pathway not only in oncogenesis but also in cognition, growth and development.

In recent years germline mutations in genes coding for other components of the RAS signalling cascade have been recognized in a group of phenotypically overlapping disorders, referred to as the neuro-cardio-facial-cutaneous syndromes or Ras-o-pathies. These disorders are characterized by variable degrees of cognitive impairment, cardiac abnormalities, facial dysmorphism, short stature, skin abnormalities and a variable cancer risk.

ES9.1
Arrays in daily practice

***C. M. A. van Ravenswaaij-Arts**;*
University Medical Centre Groningen, Department of Genetics, University of Groningen, Groningen, Netherlands.

This presentation will focus on the interpretation of array results in a routine diagnostics setting from a cytogenetic and clinical point of view. The main take-home messages will be:

1. Know your diagnostic limits. Within these limits, data should be of high quality, accurate and reproducible.
2. Define thresholds that are realistic for the array platform used and are manageable without losing too much information.
3. Be aware of the relationship of the array result with the underlying cytogenetic abnormality and with the possible mechanism of formation. This is important for the follow-up studies that should be recommended (e.g. karyotyping, FISH or array) and for counselling (e.g. recurrence risk).
4. Recognise the importance of clinical information and the use of databases.
5. Know when not to use arrays, and ensure you answer the physician's question.
6. Be aware of the increasing number of CNVs that are "risk factors" for MR/MCA, but which, on their own, may not be sufficient to cause a phenotype. This also implies that inheriting a CNV from a non-affected parent may mean it can still make a contribution to the child's phenotype.
7. Be aware that for CNVs, "*de novo*" does not automatically mean "pathogenic".
8. Be aware of unexpected findings. Pre-test counselling on the possibility of unexpected findings is important. If no national guidelines are available, local guidelines should be set up.

I will give illustrative examples for all these take-home messages.

ES9.2
Deciphering Developmental Disorders

***N. P. Carter**;*
Molecular Cytogenetics, Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

Improved diagnosis for children with developmental disorders requires the integration of several important elements. These include accurate and detailed phenotyping, reproducible and consistent application of new genomic methodologies, a comprehensive database to inform diagnosis and genetic counselling, open data sharing for additional research opportunities and a comprehensive collection of patient and parental DNAs to facilitate further disease-gene discovery. We are bringing all these elements together by direct collaboration between the 23 UK Regional Genetics Services and the Wellcome Trust Sanger Institute in the Deciphering Developmental Disorders (DDD) project (http://www.ddduk.org). The main aim of the DDD project is to improve diagnosis for children with developmental disorders within a 5-year period. This will be achieved by the systematic application of the latest microarray and sequencing methods to 12,000 children with developmental disorders and their parents from the UK recruited through the Regional Genetics Services. The new technologies will raise ethical challenges that we will address by a programme of

ethics and sociological research, working with patients and the UK genetics community. The high-resolution genomic and phenotypic data collected on this unparalleled scale will deliver accurate diagnosis for 1000s of families; a knowledge base for clinical interpretation of results from new technologies; the design of cost effective, efficient diagnostic assays; standards of good practice addressing ethical issues raised by new genomic technologies; and the opportunity for major UK research into diagnostic applications based on next generation sequencing.In his educational session, I will describe how the current DECIPHER database (https://decipher@sanger.ac.uk) can be used to inform diagnosis of developmental disorders and how the database has been adapted to provide informatics support for patient recruitment and clinical reporting of significant findings for the DDD project. Furthermore, I will describe the pragmatic approach we are taking to filtering the research findings to maximise the feedback of pertinent findings to the clinical teams while minimising the risk of feeding back incidental findings that might cause psychological harm to the patient or family.

C01.1

Landscape of somatic structural alterations in chronic lymphocytic leukemia (CLL) detected by whole-genome sequencing

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Cancer is driven by somatically genetic mutations that can be acquired gradually overtime or by single catastrophic events. Chronic lymphocytic leukemia (CLL) is one of the most frequent cancers in western countries, representing 35% of all cases of leukemia. Different genetic alterations have been identified in CLL, including chromosomal aberrations detected by cytogenetic analysis, FISH and aCGH. The limitations of these techniques do not allow the identification of the full set of somatic structural variation at molecular level. Our understanding of oncogenesis has benefited greatly from next-generation sequencing technology. Massively sequencing, in combination with computational approaches, offers the potential to carry out genome-wide screening of all sort of chromosomal rearrangements. Using a toolkit (PeSVFisher) that includes several algorithms to identify structural variations in genomic sequencing, we have analyzed whole-genome sequencing, whole-exome sequencing and SNP/CGH arrays data, to annotate the entire complement of genomic rearrangements in ten patients with CLL. By identifying read pairs that did not align correctly with respect to each other on the reference genome, we have catalogued hundreds of somatic rearrangements. Moreover, depth of coverage of whole-genome sequencing and whole-exome sequencing data provides a comprehensive catalogue of copy number variations. The analysis of these first ten CLL cases represents the first step in a comprehensive characterization of the genomic alterations at the structural level, coupled with functional analysis, of CLL in the framework of the CLL genome project to decode the genetic changes that are responsible for the development and progression of CLL.

*Equal contribution

C01.2

Exome sequencing in the identification of breast cancer predisposition genes

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High, intermediate and low penetrance genetic variants predisposing to breast cancer have been identified but account for <30% of estimated disease heritability. Our systematic resequencing of candidate genes previously identified the predisposition genes, *CHEK2, ATM, BRIP1* and *PALB2*. These genes are characterized by rare, inactivating, primarily truncating mutations that confer a relative risk of breast cancer of 2-4. It is highly plausible that there exist additional genes acting in a similar fashion and whole exome sequencing should allow their identification. However, there are significant scientific and cost constraints on such experiments.

To explore the utility of exome sequencing in gene identification in breast cancer susceptibility we have sequenced 50 familial breast cancer exomes using the Agilent whole-exome pulldown, Illumina Genome IIx sequencing and NextGENe software for data analysis. We first identified rare (<5% frequency) mutations resulting in premature protein truncation, as these have the highest likelihood of being associated with disease. Each sample contained ≥ 15 such mutations. Five were in known genes (*CHEK2* (n=3) *ATM* (n=1) and *BRCA2* (n=1)) and therefore likely to be causative. However, without prior knowledge of their role in breast cancer predisposition, recognition of these as pathogenic mutations would have been challenging. Amongst the remaining 45 samples were some interesting genes, including some with two truncating mutations which we are following up.

Our data demonstrates that exome sequencing in familial breast cancer can identify predisposition genes but that there are significant challenges in using this approach in an agnostic fashion to discover new genes.

C01.3*

The role of germline allele-specific expression of TGFBR1 in colorectal cancer predisposition

N. Segui[1], *K. N. Stevens[2], E. Guino[3], L. S. Rozek[4], V. R. Moreno[3], G. Rennert[5], G. Capellà[1], S. B. Gruber[2], L. Valle[1];*

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A previous report suggested that allele-specific expression (ASE) of *TGFBR1* confers an increased risk of colorectal cancer (CRC)[1]. Subsequent studies showed contradictory results[2-5]. Considering its potential use in the clinical evaluation of CRC susceptibility we herein perform a validation study.

While in the original study ASE was measured using SNaPshot, here we use pyrosequencing. We show that this technology is a more powerful and robust method for allelic imbalance assessment, showing reproducible and consistent results when using different allelic markers, even when high-quality RNA is not available. Blood lymphocytes and normal colon mucosae were used as source of non-tumoral biological material.

Two different populations were analyzed: 433 healthy controls and 426 CRC patients of Ashkenazi Jewish descent, and 178 CRC cases from a Caucasian-dominated population. In the Ashkenazi cohort, 26% controls and 27% patients were informative. ASE values ranged from 0.67 to 1.39 (median: 1.00) for controls and from 0.76 to 1.67 (median: 1.00) for cases (median difference -0.003; 95%CI -0.032 to 0.025; p=0.81). 50% of the Caucasian cases resulted informative. Their ASE values ranged from 0.68 to 1.40 (median: 1.01), and no significant differences were identified neither when compared with the Ashkenazi controls or with the cases. Only 25-50% of all individuals are informative for analysis, so novel approaches may be required to comprehensively determine the role of allele-specific expression of TGFBR1 in colorectal cancer.

Our results suggest that ASE of *TGFBR1* does not confer an increased risk of CRC.

C01.4*

Chromosomal, epigenetic and microRna-mediated inactivation of LRP1B, a modulator of the extracellular environment of thyroid cancer cells

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The Low density lipoprotein receptor Related Protein (LRP1B), encoding an endocytic LDLfamily receptor, is amongst the 10 most significantly deleted genes across 3,312 human cancer specimens. We undertook the present study to elucidate the mechanisms of LRP1B inactivation in cancer cells and to investigate the key roles of this lipoprotein receptor in the carcinogenesis. Here we show that LRP1B inactivation (by chromosomal, epigenetic and microRNA-mediated mechanisms) modulates the tumor microenvironment to confer cancer cells an increased invasive capacity. LRP1B displays frequent DNA copy number loss and CpG-island methylation resulting in mRNA under-expression. By using CpG-island reporters methylated *in vitro*, we found that DNA methylation disrupts a functional binding site for the histone-acetyltransferase p300 located at intron 1. We identified and validated a microRNA targeting LRP1B (miR-548a-5p) which is overexpressed in cancer cell lines as a result of 8q22 DNA gains. Restoration of LRP1B impaired *in vitro* and *in vivo* tumor growth, inhibited cell invasion and led to a reduction of soluble growth factors, cytokines and matrix metalloproteinases in the extracellular medium. These findings link DNA copy-number changes, an epigenetic transcription factor and a specific microRNA with the modulation of the extracellular medium, through LRP1B (de)regulation.

C01.5
Chromosomal instability in cancer: How does p53 guard the genome?

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Chromosomal instability, a high rate of chromosome missegregation during mitosis, is a hallmark of many human cancers and a major driving force for solid tumor progression. The mitotic checkpoint, a key surveillance mechanism to safeguard against chromosomal instability, is frequently hyperactivated in tumor cells. While we know that p53 protects against varies forms of genomic instability, how aberrant p53 signaling leads to chromosomal instability through mitotic checkpoint hyperactivation has thus far remained elusive. We find that, via induction of p21 expression and inhibition of cyclin-dependent kinase activity, crosstalk to the Retinoblastoma (Rb) pathway is critically important to prevent overexpression of the E2F target gene *Mad2*. Mad2 is a mitotic checkpoint protein whose overexpression is sufficient to hyperactivate the checkpoint and initiate aneuploid tumor formation *in vivo*. We show here that its overexpression is required to mediate the chromosomal instability phenotypes of impaired p53 and Rb pathways. In the context of aberrant Rb pathway signaling, Mad2 overexpression is required for the acquisition of chromosomal instability and anaplastic mammary tumor formation. Additionally, in a p53 mutant knock-in mouse model that develops tumors with stable, diploid genomes, the Mad2 upregulation and chromosomal instability that is caused by p21 loss can be rescued by genetic reduction of Mad2 levels. These results demonstrate that Mad2 overexpression is an essential mediator of the chromosomal instability and tumor phenotypes observed upon inactivation of the two tumor suppressor pathways that are most frequently impaired in human cancer.

C01.6
Germline gain-of-function mutations of *ALK* disrupt central nervous system development

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Neuroblastoma (NB) is a frequent embryonal tumour of sympathetic ganglia and adrenals with extremely variable outcome. Recently, somatic amplification and gain-of-function mutations of the anaplastic lymphoma receptor tyrosine kinase (*ALK,* MIM 105590) gene, either somatic or germline, were identified in a significant proportion of NB cases.

Here we report a novel syndromic presentation associating congenital NB with severe encephalopathy and abnormal shape of the brainstem on brain MRI in two unrelated sporadic cases harbouring *de novo*, germline, heterozygous *ALK* gene mutations. Both mutations are gain-of-function mutations that have been reported in NB and NB cell lines only. Electroporation of the neural tube of embryonic day 1.5 chicken embryos with a mutant *ALK* construction results in spreading of neural progenitors beyond the ventricular zone at two days post-electroporation; this phenotype is not observed when wild-type *ALK* is over-expressed. This supports the hypothesis that activating mutations in ALK disrupt neurogenesis in the central nervous system. These observations further illustrate the role of oncogenes in both tumour predisposition and normal development, and shed light on the pleiotropic and activity-dependent role of ALK in humans. More generally, missing germline mutations relative to the spectrum of somatic mutations reported for a given oncogene may be a reflection of severe effects during embryonic development, and may have to be looked for in patients with extreme phenotypes.

C02.1
Clinical and molecular findings in 91 patients with the clinical diagnosis of Cornelia de Lange Syndrome

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Cornelia de Lange Syndrome(CdLS) is characterized by dysmorphic features, growth and cognitive impairment, limb malformations and other features. Mutations in the genes NIPBL,SMC1A and SMC3 are known to cause CdLS.

91 patients were referred to us for molecular analysis of NIPBL and SMC1A.

In 64 patients we found no mutations.In 30 of them we support the clinical diagnosis CdLS, in 13 we suggest a different condition. For 21 patients insufficient clinical information was supplied.

In 27/91patients we found probably pathogenic mutations, 25 in NIPBL and 2 in SMC1A. 21 are novel mutations. 13/27patients have a mild, three an intermediate whereas six patients have a severe CdLS phenotype. In 5/27patients the clinical data was insufficient.

95% of the patients with NIPBL or SMC1A mutation show typical facial features for CdLS.All patients have mental retardation(59% mild,18% moderate,23% severe). 18% have severe malformations of the upper limbs and other 36% show typical X-ray findings. For 72% intrauterine growth retardation was reported and 67% have short stature at present. 75%of the patients are dystrophic and 94% have microcephaly. Further features are feeding difficulties(76%), cleft palate(24%), congenital heart defects(40%), kidney anomalies(12%), gastrooesophageal reflux and hearing loss.

It is of note that one SMC1A mutation is familial. The patient´s mother also shows features of CdLS.

In addition we report a case of gonadal mosaicism with two affected sibs and parents not carrying the pathogenic NIPBL mutation.

We discuss the clinical and molecular findings of 91patients with suspected clinical diagnosis CdLS and compare them with the literature.

C02.2*
Advances in phenotype-genotype correlations in Holoprosencephaly: about a European series of 645 probands.

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Holoprosencephaly (HPE) is the most common malformation of the human forebrain resulting from incomplete midline division of the prosencephalon. Three ranges of increasing severity are described: lobar, semi-lobar and alobar HPE. Other milder subtypes are part of the HPE phenotypic spectrum: Middle Interhemispheric Variant (MIH or syntelencephaly), septo-optic dysplasia and microforms that consist in facial midline defects without any HPE typical brain malformation.

Since 1996, a European network was organized from Rennes, France: data of 645 HPE probands and 699 relatives were analyzed and DNA prospectively collected.

Here we report the clinical and molecular data of the largest European series in HPE. Our cohort encompasses 51% of foetuses and 49% of children with a sex ratio (female/male) of 1,2. Facial defects, brain associated malformations, neural tube defects, extra-brain associated malformations are detailed. Mutations in the four main genes implicated in HPE (SHH, ZIC2, SIX3 and TGIF) were identified in 26% of cases. They are inherited in more than 70% of cases in SHH, SIX3, and TGIF. Instead of the other genes, they arise de novo in 70% of cases for ZIC2. Moreover rearrangements were detected in further 4% of cases by subtelomeric MLPA analysis and in further 22% in 260 HPE patients screened by array-CGH. Interestingly some probands have both a microrearrangement and a mutation in another HPE gene which adds additional support to «multiple-hit process» in HPE.

Based on statistical analyses we report new phenotype-genotype

correlations that may help define molecular analysis strategy and genetic counseling.

C02.3*

Microcephaly with simplified gyration, West syndrome and infantile diabetes linked to inappropriate apoptosis of neural progenitors

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We describe the causative gene defect in a new syndrome with primary microcephaly, infantile spasms epilepsy, diabetes and early death. Microcephaly with simplified gyration (MSG) is a rare malformation of cortical development, not always associated with severe epilepsy. Infantile onset permanent diabetes is caused by inappropriate apoptosis of pancreatic beta cells, e.g. Wolcott-Rallison syndrome (WRS).We have previously described a WRS patient with MSG suggesting abnormal apoptosis as a common mechanism. Additional families exhibiting this combination have been collected.

Genomic linkage revealed a region on chromosome 18 with a LOD score of 4.3.This corresponded to an area of overlapping homozygosity on SNP array.

Two homozygous non-conservative missense mutations in a novel gene were found in patients from two unrelated consanguineous families, but not in 300 matched controls, and were predicted as pathogenic by Poliphen-2, SIFT programs and the HOPE project. The gene involved is highly expressed in ventricular and subventricular zone of the fetal brain cortex and pancreas and is presumably implicated in regulation of apoptosis. Patient fibroblasts and cells treated with specific siRNA showed an increased susceptibility to apoptosis under stress conditions. Autopsy specimen from one patient showed increased apoptosis in the cerebral cortex. qRT-PCR revealed dysregulation of other genes in the apoptotic control system.in patient cells.

We have identified a new syndrome with MSG, epilepsy and infantile diabetes caused by novel mutation pointing to apoptosis of neural progenitors in the developing cortex as the causal mechanism. This work sheds light on the mechanisms of brain development and West syndrome.

C02.4*

Ageing in Prader-Willi syndrome: Twelve persons over the age of 50 years

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Introduction: The life expectancy of persons with Prader-Willi syndrome (PWS) has increased in recent years. Because of the paucity of reports on older persons with PWS, the natural history, the onset and type of age related problems are not well known. We will present data on physical, behavioural, psychiatric and ageing characteristics in the first cohort of individuals with PWS aged over 50 years.

Method: Twelve persons with a genetically confirmed diagnosis of PWS aged over 50 years are described (4 deletion; 8 mUPD). Data were collected through semi-structured interviews with the individuals with PWS and their main carers.

Results: Cardiovascular diseases, diabetes, dermatological and orthopedic problems were common physical complaints in elderly with PWS. Function in ADL, psychological functioning, physical functions and care dependence were substantially worse in elderly (50+) compared to the control group (18-49 yrs). 7/8 persons with mUPD had a history of psychiatric illness. Behavioural and food related problems were not less prevalent in the older individuals compared to the control group.

Discussion: Given the combination of age related physical morbidity, physical appearance, behavioural and psychiatric problems and functional decline in our cohort, we hypothesize premature ageing in elderly with PWS. Healthy ageing in PWS requires a lifespan approach that recognizes the presence, progression and consequences of specific morbidity. The knowledge of the PWS specific age related health risk factors will be used to adjust guidelines for preventive management.

C02.5

Craniofacial characteristics of Fragile X syndrome identified by dense surface and signature graph analyses

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For a disorder as common as fragile X syndrome, a hereditary form of cognitive impairment, the facial features are relatively ill defined. An elongated face and prominent ears are the most commonly accepted dysmorphic hallmarks. We analysed 3D photographs of 50 males with full FMR1 mutations using dense surface modelling (DSM) techniques and a new method that forms a directed graph with normalised faces as nodes and edges linking faces showing closest dysmorphism. We identified as yet unrecorded facial characteristics such as infra-orbital proptosis, hyperplasticity of the nasal bone-cartilage interface, mid-facial hyperplasia and increased supra-orbital height. Subsequently, we began analysis of micro-CT images of the fragile X mouse model. Results indicated reduced inter-parietal bone length and reduced length and width of the inner skull. We also found reduced lateral separation of condylar surfaces and increased mandibular height due to altered orientation of condyle, coronal and angle tip, and increased depth at anterior molars.

Thus, we have refined the facial characteristics of fragile X syndrome and have obtained a 95% discrimination rate between affected and control males. Some of the abnormalities, e.g., nasal cartilage deficiencies in patients and mid-facial effects in the mouse model, correspond in both species and suggest a common mechanism. This supports reports that FMR1, the gene silenced in the disorder, influences eye and cranial cartilage development through effects on neural crest cells and is consistent with a previous study of Bardet-Biedl syndrome where similar nasal bone and mid-facial abnormalities were linked to adverse neural crest cell behaviour.

C02.6

Epigenetic modification of the FMR1 gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056.

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Fragile X syndrome (FXS) is caused by expansion of a CGG repeat in the 5' untranslated region of the fragile X mental retardation 1 (FMR1) gene. This mutation is associated with hypermethylation

at the FMR1 promoter and subsequent transcriptional silencing. The absence of FMRP (FMR1 protein) at the synapse has many consequences, including up-regulation of metabotropic glutamate receptor 5 (mGluR5)-mediated signaling. It has been postulated that this increased mGluR5 signal may be responsible for many of the clinical manifestations observed in fragile X syndrome. mGluR5 receptor antagonists have shown promise in preclinical FXS models and in one small open-label study of FXS. We examined whether a receptor subtype-selective inhibitor of mGluR5, AFQ056, improves the behavioral symptoms of FXS in a randomized, double-blind, two-treatment, two-period, crossover study of 30 male FXS patients aged 18 to 35 years. We detected no significant effects of treatment on the primary outcome measure, the Aberrant Behavior Checklist-Community Edition (ABC-C) score, at day 19 or 20 of treatment. In an exploratory analysis, however the patients with full FMR1 promoter methylation and no detectable FMR1 messenger RNA improved, as measured with the ABC-C, significantly more after AFQ056 treatment than with placebo ($P < 0.001$). If confirmed in larger and longer-term studies, these results suggest that blockade of the mGluR5 receptor in patients with full methylation at the FMR1 promoter may show improvement in the behavioral attributes of FXS.

C03.1*

Mutations in multiple components of the pre-replication complex cause microcephalic primordial dwarfism and Meier-Gorlin syndrome

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Microcephalic primordial dwarfism is an autosomal recessive group of conditions with extreme growth failure, characterised by intrauterine growth retardation, short stature and microcephaly. Several genes involved in fundamental cellular processes have been identified for these disorders: PCNT in MOPD II ; ATR , CEP152 in Seckel syndrome; but none previously for Meier-Gorlin syndrome.

Here, we report the identification of five further genes encoding components of the pre-replication complex. Homozygosity mapping in a Saudi Arabian family with an uncategorised form of microcephalic primordial dwarfism established a novel locus at 1p32.3. Subsequent sequencing identified biallelic missense ORC1 mutations in 4 families with microcephalic dwarfism from a cohort of 170 cases. We found that replication licensing was impaired in Orc1 patient cell lines and cell cycle progression slowed, in keeping with ORC1's role, (along with ORC2-6, CDC6 and CDT1), as a component of the Pre-Replication Complex that licenses replication initiation. Furthermore, developmentally, Orc1 depletion substantially impaired zebrafish embryo growth.

Extending our studies to patients with Meier-Gorlin syndrome, in which primordial dwarfism is accompanied by absent or hypoplastic patellae and microtia, we then identified biallelic missense/truncating mutations in ORC1, and through further sequencing multiple mutations in other preRC complex components, ORC4, ORC6, CDT1 and CDC6. We therefore implicate the pre-replication complex as a whole in the developmental regulation of human growth, and surprisingly show that disruption of its function is associated with a distinct and broad spectrum of developmental abnormalities.

Bicknell, Walker et al. Nat.Genet, in press;
Bicknell, Bongers et al. Nat.Genet, in press.

C03.2

Exome sequencing identifies truncating mutations in NOTCH2 as a cause of Hajdu-Cheney syndrome

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Hajdu-Cheney syndrome is a rare skeletal disorder characterized by the association of facial anomalies, radiological findings (acro-osteolysis, general osteoporosis, insufficient ossification of the skull) and periodontal disease (premature loss of permanent teeth). Although most cases are sporadic, transmission in a few families suggests autosomal dominant inheritance. We sequenced the exomes of six unrelated patients and identified nonsense and frameshift heterozygous mutations in *NOTCH2* for five of them. The five mutations are invariably located within the last 3' coding exon of *NOTCH2*, distal to the ankyrin repeats and NLS necessary for the transcriptional activity of Notch2 intracellular domain, while deleting the PEST domain. These results suggest that mutant mRNA products escape nonsense-mediated decay and that truncated *NOTCH2* proteins act in a gain-of-function manner. This mutational mechanism also suggests that decreasing Notch2 signaling using selective gamma-secretase inhibitors may have therapeutic interest in this severe and progressive disease. Finally, our findings establish an important role for Notch2 signaling in bone homeostasis that may notably be relevant to better understanding of mechanisms leading to osteoporosis.

C03.3

Exome sequencing identifies truncating mutations in human SERPINF1 in autosomal-recessive Osteogenesis Imperfecta

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Osteogenesis imperfecta (OI) is a heterogeneous genetic disorder characterized by bone fragility and susceptibility to fractures after minimal trauma. After mutations in all known OI genes had been excluded by Sanger sequencing, we applied next-generation sequencing to analyze the exome of a single individual with a severe form of the disease whose parents are second cousins. A total of 26,922 variations from the human reference genome sequence were subjected to several filtering steps. In addition, we extracted the genotypes of all dbSNP130-annotated SNPs from the exome sequencing data and used these 299,494 genotypes as markers for the genome-wide identification of homozygous regions. A single homozygous truncating mutation, affecting SERPINF1 on chromosome 17p13.3, remained that was embedded into a homozygous stretch of 2.99 Mb. The mutation was also homozygous in the affected brother of the index patient. Subsequently, we identified homozygosity for two different truncating SERPINF1 mutations in two unrelated patients with OI and

parental consanguinity. All four individuals with SERPINF1 mutations have severe OI. Fractures of long bones and severe vertebral compression fractures with resulting deformities were observed as early as in their first year of life. Collagen analyses with cultured dermal fibroblasts displayed no evidence for impaired collagen folding, posttranslational modification, or secretion. SERPINF1 encodes pigment epithelium-derived factor (PEDF), a secreted glycoprotein of the serpin superfamily. PEDF is a multifunctional protein and one of the strongest inhibitors of angiogenesis currently known in humans. Our data provide genetic evidence for an involvement of PEDF in human bone homeostasis.

C03.4*

Mutations in lectin complement pathway genes COLEC11 and MASP1 cause 3MC syndrome

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3MC syndrome has been proposed as a unifying term encompassing the overlapping Carnevale, Mingarelli, Malpuech and Michels syndromes. These rare autosomal recessive disorders exhibit a spectrum of developmental features, including characteristic facial dysmorphism, cleft lip and/or palate, craniosynostosis, learning disability and genital, limb and vesicorenal anomalies. Here we studied eleven families with 3MC syndrome and identified two mutated genes, COLEC11 and MASP1, both of which encode proteins in the lectin complement pathway (collectin kidney 1 (CL-K1) and MASP-1 and MASP-3, respectively). CL-K1 is highly expressed in embryonic murine craniofacial cartilage, heart, bronchi, kidney and vertebral bodies. Zebrafish morphants for either gene develop pigmentary defects and severe craniofacial abnormalities. Finally, we show that CL-K1 serves as a guidance cue for neural crest cell migration. Together, these findings demonstrate a role for complement pathway factors in fundamental developmental processes and in the etiology of 3MC syndrome.

C03.5

Genetic deficiency of tartrate-resistant acid phosphatase (TRAP) associated with skeletal dysplasia, cerebral calcifications and autoimmunity: delineation of a new defect in a lysosomal enzyme

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Vertebral and metaphyseal chondrodysplasia, spasticity with cerebral calcifications, and strong predisposition to autoimmune diseases such as systemic lupus erythematosus and autoimmune cytopenias are the hallmarks of the genetic disorder Spondyloenchondrodysplasia (SPENCD; MIM 271550). We mapped a locus to chromosome 19p13 and identified ten different mutations in the ACP5 gene in 14 patients aged from 3 to 63 years.

ACP5 encodes tartrate-resistant phosphatase (TRAP), a lysosomal enzyme that is expressed predominantly in macrophages, osteoclasts and dendritic cells. The mutations abolish enzyme function in the serum and cells of affected individuals. Phosphorylated osteopontin (p-OPN), a protein involved in bone reabsorption and in immune regulation, accumulates in serum, urine and cells cultured from TRAP-deficient individuals. Patient-derived dendritic cells exhibit an altered cytokine profile and are more potent than matched control cells in stimulating allogeneic T cell proliferation in mixed lymphocyte reactions. Thus, TRAP deficiency leads to (1) a defect in bone remodeling, particularly at growth plates, and (2) accumulation of phosphorylated osteopontin, a potent pro-inflammatory cytokine, leading to interferon release and autoimmunity.

The discovery of TRAP deficiency as the basis of SPENCD discloses new therapeutic options such as hematopoietic stem cell transplantation, enzyme replacement therapy, or pharmacologic modulation of the OPN-IFN axis. The findings also shed new light on the role of osteopontin and its regulation by tartrate-resistant acid phosphatase in the pathogenesis of common autoimmune disorders.

C03.6*

De novo nonsense mutations of ASXL1 cause Bohring-Opitz (Oberklaid-Danks) syndrome

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Bohring syndrome is characterized by severe intellectual disability, distinctive facial features and multiple congenital malformations. Whole exome sequencing and follow-up by Sanger sequencing revealed heterozygous de novo nonsense mutations in the additional sex combs like 1 gene (ASXL1) in seven patients with Bohring syndrome. ASXL1 belongs to the Polycomb group (PcG) and trithorax complexes family. This family of genes is required for maintenance of both activation and silencing of Hox genes, which determine segmental identity in the developing embryo. Somatic mutations in ASXL1 have previously been reported with leukemia and myelodysplactic syndrome. Thus, our findings suggest a new link between severe developmental disorders and cancer.

C04.1

A Unifying Genetic Model for Facioscapulohumeral Muscular Dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is characterized by progressive and often asymmetric weakness and wasting of facial, shoulder girdle and upper arm muscles. FSHD is most often caused by contraction of the D4Z4 macrosatellite repeat array, but only when the repeat array is localized on a specific permissive genetic background of chromosome 4q. Repeat contractions non-permissive 4q or 10q chromosomes do not cause FSHD. D4Z4 repeat contractions are associated with local chromatin relaxation and transcriptional upregulation of the retrotransposed gene *DUX4* in FSHD myotubes only.

We performed detailed D4Z4 sequencing in permissive and non-permissive haplotypes and identified specific single nucleotide polymorphisms (SNPs) in the polyadenylation signal of *DUX4*. While permissive chromosomes carry a typical polyadenylation signal for *DUX4*, non-permissive chromosomes have sequence variants that would predict the *DUX4* polyA signal to be non-functional. Indeed, transfection studies revealed that *DUX4* transcripts are efficiently polyadenylated and more stable when expressed from permissive chromosomes. These findings suggest that FSHD arises through a toxic gain of function attributable to the stabilized *DUX4* transcript. In addition to the SNPs affecting the polyadenlyation signal other SNPs are currently being investigated for their role in *DUX4* mRNA processing and stability. In controls *DUX4* is highly expressed in the germ line and then is epigenetically silenced in somatic tissues. In FSHD, the combination of inefficient D4Z4 chromatin silencing and stabilization of *DUX4* mRNAs results in inappropriate DUX4 protein expression in muscle cells. Therefore, FSHD represents the first human disease caused by the inefficient repression of a retrogene array.

C04.2*

High-resolution breakpoint mapping of 10 regulatory and 32 *FOXL2* encompassing deletions in BPES using targeted microarrays

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Blepharophimosis syndrome (BPES) is a rare autosomal dominant developmental disorder characterized by a complex eyelid malformation associated or not with ovarian dysfunction. Deletions encompassing the *FOXL2* gene and regulatory deletions located outside its transcription unit represent 12% and 5% of molecular defects respectively. In total we identified 32 *FOXL2* encompassing and 10 regulatory deletions. The deletion size and breakpoint locations are highly variable, suggesting absence of a recombination hotspot. To unravel the mechanisms underlying these rearrangements, we aimed to determine the precise locations of the breakpoints. A second purpose was to identify genotype-phenotype correlations for extra-ocular features. We used high-resolution tiling arrays to fine-map the breakpoints. For 27 deletions, both breakpoints could be fine-mapped, allowing junction PCRs. Of these, six deletions could be delineated at the nucleotide level so far. At least two out of six deletions are caused by a recombination-based mechanism using LINE and *Alu* elements as recombination substrates. With regard to genotype-phenotype correlations, an *ATR* encompassing deletion was found in five of eight BPES patients with reported microcephaly. Interestingly, in two patients with joint abnormalities the *SOX14* gene was located within the deletion, potentially implicating this gene in the pathogenesis of this extra-ocular feature. In the largest series of *FOXL2* encompassing and regulatory deletions in reported, high-resolution breakpoint mapping was used to gain more insights into the mechanisms underlying the deletions, and to correlate deletion size and breakpoint location to extra-ocular features.

C04.3

Non-coding, regulatory mutation implicates the HCFC1 gene in non-syndromic intellectual disability.

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Recent systematic X chromosome exon re-sequencing and high resolution copy number profiling on a set of 208 families resolved only ~50% of these. In one unresolved family, known as MRX3 (J Med Genet, 28, 372-7; 1991), we excluded the presence of a mutation in 99.5% of the coding sequence in the linkage interval by massively parallel (MPS) and Sanger sequencing. We postulated a non-coding mutation might be disease causing and from 15 such (unique) variants we investigated one, chrX(hg18) 152890455 A>G, in further detail. We show, that this intergenic nucleotide change unidirectionally affects the expression of the HCFC1 gene. This variant occurs in one of six consensus binding sites of the YY1 transcription factor. We examine its effect by EMSA and luciferase reporter assays and show that YY1 binding is abolished. HCFC1 forms a complex (among others) with the MLL family of H3K4 methyltransferases that is important in cell cycle regulation. We also show further downstream effect of HCFC1 de-regulation on the E2F1 transcription factor and as such putatively linking HCFC1 de-regulation to p53 regulated neurogenesis. Interestingly, YY1 itself has recently been found mutated in a sporadic case with ID (Nat Genet, 42(12):1109-12, 2010). Our data show that patients with MECP2 (and also HCFC1) duplications do not have their HCFC1 mRNA up regulated. Currently ~86% of all disease causing mutations are in protein coding regions, however, this may represent ascertainment bias, which can now be addressed at least partly by the application of MPS technology.

C04.4

Distinct effects of allelic NFI-X mutations on nonsense-mediated mRNA decay engender either a Sotos-like or a Marshall-Smith syndrome

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Using a combination of array comparative genomic hybridization and a candidate gene approach, we identified nuclear factor I/X (NFI-X) deletions or nonsense mutations in three sporadic cases of a Sotos-like overgrowth syndrome with advanced bone age, macrocephaly, developmental delay, scoliosis and unusual facies. Unlike the aforementioned human syndrome, Nfi-x-deficient mice are unable to gain weight and die in the first three postnatal weeks, while they also present with a spinal deformation and decreased bone mineralisation. These features prompted us to consider NFI-X as a candidate gene for Marshall-Smith syndrome (MSS), a severe malformation syndrome characterized by failure to thrive, respiratory insufficiency, accelerated osseous maturation, kyphoscoliosis, osteopenia and unusual facies. Distinct frameshift and splice NFI-X mutations creating premature translation termination codons that escaped nonsense-mediated mRNA decay (NMD) were identified in nine MSS subjects. NFI-X belongs to the Nuclear factor one (NFI) family of transcription factors, but its specific function is presently unknown. We demonstrate that NFI-X is normally expressed prenatally during human brain development and skeletogenesis. These findings demonstrate that allelic NFI-X mutations trigger distinct phenotypes, depending specifically on their impact on NMD.

C04.5*

Revertant somatic mosaicism in Dyskeratosis Congenita by mitotic recombination.

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Somatic mosaicism due to reversion of a pathogenic allele to wild type is an intriguing phenomenon. We have observed reversion by mitotic recombination of a mutation in *TERC* (telomerase RNA component) in a family affected by Dyskeratosis Congenita (DC). DC is a disorder of telomere biology and is characterized by mucocutaneous abnormalities and dystrophic nails. Persons affected by DC are at risk for bone marrow failure, cancer and lung fibrosis.

We identified a deletion in *TERC* in the proband of a family with autosomal dominant DC. Segregation of the mutation was confirmed in all affected relatives by sequence analysis on peripheral blood DNA. In two affected brothers, discrepancies in the peak heights suggested overrepresentation of the wild-type allele, whereas the mutation had remained heterozygous in tissues other than blood. Therefore, this mosaicism in peripheral blood must result from somatic reversion of the mutated allele to normal. Neither one of these patients developed bone marrow failure. SNP array analysis on blood DNA from the brothers showed acquired uniparental disomy of chromosome 3q, including *TERC*. In one of these two patients, two independent events of mitotic recombination on chromosome 3 were observed, which were both present in different blood cell lineages of this person. Therefore, the reversion events must have occurred in non-lineage specific haematopoietic precursor cells.

In conclusion, we have found recurrent revertant mosaicism in a family with DC. This finding is important for improving diagnostic testing and understanding the variable phenotype of DC.

C04.6

Mutations in RAD21 as a cause of a new cohesinopathy

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Cornelia de Lange syndrome (CdLS) is a dominantly inherited multisystem developmental disorder that includes distinctive facial features, growth retardation, limb defects and variable cognitive delay. Mutations in *NIPBL* have been identified in approximately 75% of patients with severe classical CdLS but have been found in a far lower percentage of patients with mild or atypical features. NIPBL is required for the establishment of sister chromatid cohesion through the loading of Cohesin, a multimer consisting of SMC1 and SMC3 and the clasp proteins, RAD21 and STAG. Following the identification of *NIPBL*, mutations in *SMC1A* and *SMC3* were detected in a small percent of patients. These patients have a milder phenotype without major structural anomalies, although all have significant mental retardation. By mutation-screening of several genes encoding cohesins or associated proteins in a cohort of 180 mutation-negative individuals, two *de-novo RAD21*-mutations in patients with features that diverge from classical CdLS were identified. We have measured the expression and activity of these missense-mutations in protein-interaction, DNA-damage and zebrafish developmental-assays. In short, one of these RAD21-mutations which increases binding to STAG2, appears to function in a dominant-negative manner and results in a more severe phenotype than the other loss-of-function mutation, which alters binding to SMC1. Cohesinopathies comprise a broadening spectrum of pediatric developmental disorders. We will discuss the current understanding of mechanisms by which Cohesin mutations may alter transcriptional profiles to result in multiple variant developmental phenotypes and speculate on clinical features that appear to be unique and common between different "disorders of Cohesin".

C05.1

Novel Method to Estimate the Phenotypic Variation Explained by Genome Wide Association Studies Reveals Large Fraction of the Missing Heritability

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Genome-wide association studies (GWAS) are conducted with the promise to discover novel genetic variants associated with diverse traits. For most traits, associated markers individually explain just a modest fraction of the phenotypic variation, but their number can well be in the hundreds. We developed a maximum likelihood method that allows us to infer the distribution of associated variants even when many of them were missed by chance. Compared to previous approaches, the novelty of our method is that it (a) does not require having an independent (unbiased) estimate of the effect sizes; (b) makes use of the complete distribution of P values while allowing for the false discovery rate; (c) takes into account allelic heterogeneity and the SNP pruning strategy. We then applied our method to the latest GWAS meta-analysis results of the GIANT consortium. It revealed that while the explained variance of genome-wide (GW) significant SNPs is around 1% for waist-hip ratio (WHR), the observed P values provide evidence for the existence of variants explaining 10% (CI=[8.5%-11.5%]) of the phenotypic variance in total. Similarly, the total explained variance likely to exists for height is estimated to be 29% (CI=[28%-30%]), three times higher than what the observed GW significant SNPs give rise to. This methodology also enables us to predict the benefit of future GWA studies that aim to reveal more associated genetic markers via increased sample size.

C05.2

Accurate prediction of a minimal region around a genetic association signal that contains the causal variant

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In recent years, genome wide association studies have been very successful in identifying loci for complex traits. However, typically these findings involve noncoding and/or intergenic SNPs without a clear functional effect that do not directly point to a specific gene. Hence, the next challenge is to identify the causal variant responsible for the association signal. Typically the first step is to identify all genetic variation in the locus region by, for example, resequencing a large number of case chromosomes. Once all variants are identified, the causal one needs to be ascertained in further functional studies. Because the experimental follow up can be laborious, restricting the number of variants to be scrutinized would yield a great advantage. Therefore, an objective method for choosing the size of the region to be followed up would be highly valuable. Here we propose a simple method to call the minimal region around a significant association peak that is very likely to contain the causal variant. We model Linkage Disequilibrium (LD) in cases from the observed single SNP association signals and predict the location of the causal variant by quantifying how well this relationship fits the data. Simulations showed that our approach identifies genomic regions of on average ~50 kb with up to 90% probability to contain the causal variant. We apply our method to two genome-wide association datasets and localize both the functional variant REP1 in the α-synuclein gene that conveys susceptibility to Parkinson's Disease and the APOE gene involved in susceptibility to Alzheimer's Disease.

C05.3*

Anaylsis of 150 exomes using an automated analysis pipeline

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Enrichment techniques for targeted sequencing of coding regions are currently applied to identify rare variants. We developed a pipeline to analyze exome sequencing data. The pipeline is a combination of

Perl scripts and public available software packages (BWA, SAMtools). It calculates quality metrics and performs read alignment to the reference sequence, variant calling, variant annotation and selection of candidate variants according to the genetic model. Variants are stored in a database, which allows user queries through a web interface and enables pedigree or gene based searches. Exomes deposited in the database can be used as controls.
We applied the analysis pipeline to ~150 exomes. From an average of ~7-10 GB of aligned sequence, the pipeline calls ~16,000-20,000 coding variants. Approximately 7,500-8,500 of these are non-synonymous variants of which ~700-800 not present in dbSNP (version 132). Depending on the number of affected individuals investigated and the underlying inheritance model, we are able to confine this list to 1-10 putatively disease causing variants.
We compared our results with known disease causing recessive mutations in 438 genes obtained from the Human Gene Mutation Database (HGMD). We quantified the amount of new, putatively deleterious variants and assessed the frequency of literature-annotated disease mutations. We identified 16 new heterozygous nonsense variants. 174 of HGMD annotated mutations (~0.85%) had a frequency of >5% in our samples. After subtracting these mutations with an unlikely high frequency, the carrier burden of recessive mutations from HGMD in these genes is between 0 and 9 per individual (average 3.12).

C05.4*

Identity-By-Descent Filtering of Exome Sequence data for Disease-Gene Identification in Autosomal Recessive and X-Linked Disorders

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Next-Generation Sequencing (NGS) and exome-capture technologies are currently revolutionizing the way geneticists screen for disease-causing mutations in rare Mendelian disorders. Current analysis strategies involve intersection filtering, restriction of candidates to sequence variants predicted to be deleterious, searching for de novo mutations in trios, and using linkage analysis to filter out exome sequences from chromosomal regions that do not demonstrate linkage. None of the above mentioned analysis strategies are well suited for identification of disease genes in small families segregating autosomal recessive (AR) or X-linked (XL) diseases.
Here, we present an algorithm that can be used to narrow down the candidate regions in exome sequences of affected siblings of consanguineous or non-consanguineous parents in AR disorders. Our algorithm uses a non-homogeneous hidden Markov model (HMM) that employs local recombination rates to identify chromosomal regions that are identical by descent (IBD=2) in children of consanguineous or non-consanguineous parents solely based on genotype data of siblings derived from high-throughput sequencing platforms. Using simulated and real exome sequence data, we show that our algorithm is able to reduce the search space for the causative disease gene to a fifth or a tenth of the entire exome. We have used our algorithm successively on real data to identify disease genes including the PIGV gene as the cause of Mabry syndrome (Krawitz et al., Nat Genet. 2010,42:827-9). Here, we will present the algorithm as well as a recent modification of the algorithm for XL diseases.

C05.5

Genome-Wide Association Meta-Analysis of Total Brain Volume: Results from the ENIGMA Consortium

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There is evidence that differences of brain volume, either global or regional, are related to their specific functioning and to more global cognitive processes. Our overall aim is to identify common genetic variants that modify the risk for psychiatric disorders based on the identification of genetic variants involved in the variance of the volume of brain structures in healthy individuals. Mean total brain volume was automatically segmented in T1-weighted MRI scan using FreeSurfer. 16 groups worldwide have now pooled their imaging genomics data from a total of >7000 subjects. Genetic homogeneity of the samples was assessed and genotypes were imputed using the HapMap III reference panel, and associations were tested via dosage of each imputed SNP (accounting for kinship in family-based samples). After quality control, results files and summary statistics were pooled for meta-analysis. By February 2011, 16 groups had uploaded GWAS results in >7000 subjects. Association conducted at ~1.3 million autosomal SNPs showed that none of the individual sites contained genome-wide significant GWAS results (p<10^-8). Conversely, meta-analysis of the data from the 16 studies yielded genome-wide significant p-values for total brain volume. To date, neuroimaging genetics has largely employed candidate gene, GWAS, or image-wide GWAS analyses in samples < 1000 subjects. This leads to a lack of power to detect significant effects while controlling the false positive rate. Our collaborative ENIGMA pilot project on total brain volume shows the utility of data sharing and meta-analysis, and the value of large sample sizes for genetic analysis of brain measures.

C05.6*

The influence of genetic variations on regulatory modules in the liver.

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Deciphering the influence of genetic variants on regulatory modules, and the correlation between such regulatory variants and phenotype (e.g. disease) has been the subject of extensive research in recent years. Here, we present a novel approach to this problem, which entails analyzing modules of genes, rather than studying SNP-gene connections independently. First, we search pair-wise connections between transcripts whose levels are co-associated to the same SNP. Second, we combine these pairs into modules that share a regulating SNP. Third, we assign a confidence score to each module. Finally, we split the samples in each module according to the genotype of the main SNP and find the best allele specific SNPs that explain the expression of the largest group of genes in each subset of samples. We applied our method to data on genetic and gene expression in the liver across 371 samples by Merck. We detect 129,109 pairs of transcripts that are jointly associated to the same SNP as their most significant regulator. These make up 10,354 modules, 518 including 10 transcripts or more. These are significantly more and larger modules than in permuted data (2,322+-208, 220+-42 of size 10 or more). We quantified the confidence in a module as a likelihood score, and prune a subset of 114 modules with FDR<0.02. We observe similar annotations of modules from two sources of information: the enrichment of a module in gene subsets and locus annotation of the genetic variants. This provides a validation of our methodology.

C06.1

Identification of recent admixture in an Indian population of African ancestry: prospects for complex disease mapping

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Identification and study of genetic variation in recently admixed populations not only provides insight into historical population events but is also a powerful approach for mapping disease genes. We studied a population of African-Indian(AI) origin which is believed to be the descendants from the Bantu speaking parts of Africa and have been residing in the western part of India for nearly >500years. We have carried out this study using a common set of 18534 autosomal markers; common between 26 Indian populations (50 K Affymetrix array), populations in the Human Genome Diversity Project (HGDP) and HapMap populations. Principal component analysis clearly revealed that the African-Indian population derives its ancestry from West-African as well as Indo-European speaking Northern and North-West Indian population(s). STRUCTURE and ADMIXTURE analysis revealed that, overall the AI population derive 58.7% of their genomic ancestry from their African past with very little inter-individual ancestry variation (8.4%). The extent of linkage-disequilibrium also reveals that the admixture event has been recent. Functional annotation of genes encompassing the ancestry informative markers revealed

significant enrichment of biological processes related to ion channel activity from the Indian ancestry. We briefly examine the implications of determining the genetic diversity of this population which could provide opportunities for possible studies using admixture mapping.

C06.2
Identification of loci governing phenotypic traits in dog breeds highlights disease genes of relevance to human health

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The dog population is composed of more than 400 breeds; each one is a genetic isolate with unique phenotypic characteristics. Dogs tend to suffer from the same range of diseases as humans, but the genetic complexity of these diseases within a breed is reduced as a consequence of intense selection and inbreeding.

In this study, we performed a comprehensive analysis using multiple test statistics to identify regions under strong selection in 518 dogs from 46 diverse breeds using a newly developed high-density genotyping canine array consisting of >170,000 evenly spaced SNPs. We first identified 44 genomic regions exhibiting extreme differentiation across multiple breeds. Genetic variation in these regions correlates with variation in several phenotypic traits that vary between breeds, and we identify novel associations with both morphological and behavioural traits. We next scanned the genome for signatures of selective sweeps in single breeds. These scans identify hundreds of regions strongly enriched for developmental genes and genes implicated in diseases. We characterised one highly differentiated region associated with body size and ear morphology, using high-throughput sequencing to provide a list of variants that may directly affect these traits.

In this study, we provide a detailed description of genomic regions under strong selection in the canine genome, including many linked to extreme phenotypic variation. We hypothesize that some of these regions will also contain canine disease genes of comparative relevance to human health.

C06.3
Increased number of microRNA target sites in genes encoded in CNV regions. Evidence for an evolutionary genomic interaction?

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MicroRNAs (miRNAs) and Copy Number Variations (CNVs) are two newly discovered genetic elements that have revolutionised the field of molecular biology and genetics. By performing *in silico* whole genome analysis we demonstrate that both the number of miRNAs that target genes found in CNV regions as well as the number of miRNA binding sites are significantly higher than those of genes found in non-CNV regions. Analysis of human and chimpanzee genome demonstrates that the number of microRNAs targeting a gene, increase after the formation of CNVs since genes in CNVs formed in human lineage are targeted by a significantly higher number of microRNAs than the genes found within CNVs in chimpanzee, for which data are available. Collectively these results suggest that miRNAs may have acted as equilibrators of gene expression during evolution, in an attempt to regulate aberrant gene expression and to increase the tolerance to genome plasticity.

C06.4
Loss and gain of function in human *SERPINB11:* an example of a gene under selection on standing variation, with implications for host-pathogen interactions

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Serine protease inhibitors (SERPINs) are crucial in the regulation of diverse biological processes including inflammation and immune response. *SERPINB11*, located in the 18q21 gene cluster, is a polymorphic gene/pseudogene coding for a non-inhibitory SERPIN. This study sought a better understanding of the evolutionary history of *SERPINB11*, with special focus on evaluating the adaptive signature identified in Africans in a genome-wide scan for selection. Our study comprised the resequencing of *SERPINB11* in 20 Yorubans and the analysis of primate orthologous sequences. We identified a full-length *SERPINB11* variant encoding a non-inhibitory SERPIN as the putative candidate of selection, probably driven to higher frequencies by an adaptive response using preexisting variation. In addition, we detected contrasting evolutionary features for *SERPINB11* in primates: While primate phylogeny as a whole is under purifying selection, the human lineage shows evidence for positive selection in a few codons, all associated with the active *SERPINB11*. According to comparative modeling studies, positively selected codons are suggested to reduce SERPINB11's ability to undergo the typical conformational changes of inhibitory SERPINs - suggesting that it is evolving towards a new function in humans. The recognition of significant correlations between *SERPINB11* variants and pathogen richness has led us to propose a selective advantage through host-pathogen interactions, which may be linked to an adaptive response combating the emergence of infectious diseases during the Neolithic. This work represents the first description of a resurrected gene in humans, and is likely to represent an example of selection on standing variation triggered by drastic ecological shifts.

C06.5
Remarkably little homozygosity in first generation mixed race individuals

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Long runs of homozygosity (ROH) are ubiquitous features of human genomes. There is, however, wide variation in the number and length of ROH across individuals and populations due to their diverse demographic histories: sub-Saharan African populations are the least homozygous and Native South Americans the most. Using genomic analysis we here demonstrate the remarkable lack of homozygosity in mixed race individuals: first generation (F1) West African x European individuals are the least homozygous of all, with an order of magnitude fewer ROH than commonly observed in other populations. Their parents may not share ancestors after the Out of Africa movement, ~70,000 years ago. Populations may be ranked by increasing mean number of ROH: (1) F1 West African x European; (2) F1 Afro-Caribbean x European; (3) Afro-Caribbean; (4) sub-Saharan African (excluding hunter-gatherers), F1 European x East African, F1 European x East Asian, Mauritian and Seychellois; (5) F1 European x South Asian; (6) F2 Eurasian mixed race individuals; (7) other Eurasians; and (8) Native North Americans. Afro-Caribbeans have on average ~20% European ancestry, and these haplotypes generate ROH in offspring with Europeans. F1 European x East Asian individuals carry a similar complement of ROH to sub-Saharan Africans, emphasising the great genetic diversity in Africa. The variance in the number of ROH was highest for Native North Americans: NROH was strongly inversely correlated with the large and variable genomic estimates of European admixture. The reduction in genome-wide homozygosity in mixed race individuals will reduce their risk for diseases with a recessive genetic component.

C06.6*

Microsatellite choice and Y chromosome variation: attempting to select the best STRs to date human Y chromosome lineages

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Recently the debate on the origins of the major European Y chromosome haplogroup R-M269 has reignited, and opinion has moved away from Paleolithic origins to the notion of a younger Neolithic spread of these chromosomes from the Near East. We investigate the young, STR-based Time to the Most Recent Common Ancestor estimates proposed so far for R-M269 related lineages and find evidence for an appreciable effect of microsatellite choice on age estimates. We further expand our analysis to include a worldwide dataset of over 60 STRs which differ in their molecular attributes. This analysis shows that by taking into account the intrinsic molecular characteristics of Y chromosome STRs, one can arrive at a more reliable estimate for the age of Y chromosome lineages. Subsequently, we suggest that most STR-based Y chromosome dates are likely to be underestimates due to the molecular characteristics of the markers commonly used, such as their mutation rate and the range of potential alleles that STR can take, which potentially leads to a loss of time-linearity. As a consequence, we update the STR-based age of important nodes in the Y chromosome tree, showing that credible estimates for the age of lineages can be made once these STR characteristics are taken into consideration. Finally we show that the STRs that are most commonly used to explore deep ancestry are not able to uncover ancient relationships, and we propose a set of STRs that should be used in these cases.

C07.1

Impact of mtDNA mutations on mtDNA segregation throughout human oogenesis

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Mitochondrial DNA (mtDNA) mutations cause a wide range of serious diseases with high transmission risk and maternal inheritance. Owing to the absence of therapy, couples at risk to transmit such disorders commonly ask for prenatal (PND) and preimplantation diagnosis (PGD). The lack of data regarding heteroplasmy distribution throughout oogenesis, however hampers these procedures.

We tracked the segregation of 5 mtDNA mutations, namely m.3243A>G, m.8344T>G m.8993T>G, m.9185T>C and m.10197G>A responsible for MELAS, MERRF, NARP or Leigh syndrome during oogenesis, by quantifying the corresponding mutant loads in 6 carriers, their 14 oocytes, 53 first polar bodies (PB1), and 65 early embryos thought to estimate of the mature oocyte mutant load.

Our data indicate that

1/ mtDNA segregation is governed by random genetic drift for m.3243A>G throughout the whole oogenesis.

2/ the size of the bottleneck operating during oogenesis i) is likely to depend on the mutation type, and ii) is individual-dependent for m.3243A>G.

3/ while the absence of mutation constantly correlates between PB1 and the corresponding oocyte/embryo, mutant load may vary significantly (+30%) between these 2 clonally-derived daughter cells, irrespective of the mutation type, contrary to the data drawn from mouse models.

4/ a selection event against high mutant load oocytes might occur during meiosis.

5/ there is no correlation between the PB mutant load and the rate of cleavage, suggesting that oocyte fertilization and early embryonic development are not markedly affected by mtDNA mutations.

These data have obvious consequences on genetic counseling and PND/PGD procedures in mtDNA inherited disorders.

C07.2

Pre-implantation genetic diagnosis offers a fair chance of having unaffected offspring for mitochondrial DNA disorders

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Oxidative phosphorylation disorders due to maternally inherited homoplasmic or heteroplasmic mtDNA mutations affect approximately 1/5,000 individuals. The mutation level of transmitted heteroplasmic mtDNA point mutations varies significantly between embryos due to a segregational bottleneck. Prenatal (PND) or pre-implantation genetic diagnosis (PGD) of mtDNA disorders is complicated by the inability to accurately predict the threshold of clinical expression for most heteroplasmic mtDNA mutations. Here we tried to define a minimal mutant level below which the chance for an embryo of being affected is acceptably low, irrespective of the exact mtDNA point mutation. A systematic review was performed on muscle mutant levels of 159 different heteroplasmic mtDNA point mutations derived from 327 unrelated patients or pedigrees, excluding three overrepresented mtDNA mutations. We generated a distribution of mutation levels of all affected individuals (n=195) and their unaffected maternal relatives (n=19) from 137 pedigrees with a familial mtDNA mutation, and predicted the risk of being affected given a varying mutant level. This prediction required an assumption of overall prevalence of affected status in familial pedigrees. For familial mutations, little difference in mean muscle mutant level was observed between probands and affected maternal relatives and between affected individuals with a tRNA-versus protein-coding mutation. The overall prevalence of affected status in familial pedigrees was estimated as 0.477. A 95% or higher chance of being unaffected was associated with a muscle mutant level of 18% or less. PGD provides carriers of heteroplasmic mtDNA point mutations the opportunity to substantially increase the chance of having healthy offspring.

C07.3

Multiplex massively parallel sequencing for noninvasive prenatal diagnosis

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Blood plasma of pregnant women contains circulating fetal DNA, with great potential for non-invasive detection of fetal chromosomal aneuploidies. We have explored the possibility for multiplex massively parallel sequencing to detect such fetal chromosomal aneuploidies.

From three women, carrying euploid fetuses (46,XY [N=1], 46,XX [N=2]) blood samples were drawn prior to invasive procedures and from five women, carrying aneuploid fetuses (47,XX,+21; 47,XY,+21; 47,XXX; 47,XX,+13; 47,XY,+18) blood samples were drawn after invasive procedures. Samples were taken at various gestational ages, ranging from 12^{+4} to 19^{+6}. DNA was isolated from 3.5-8 mL of plasma using either a QIAamp DSP Mini Blood Kit or a QIAamp Circulating Nucleic Acid Kit. Sequencing libraries were prepared individually and DNA was multiplexed after ligation of an unique sequencing barcode. Sequencing was performed on a SOLiD™ 4 system. Next, the sequence reads were mapped to the human reference genome (hg19) and quantified according to their genomic location. Subsequently, ζ-scores per chromosome were calculated as described elsewhere. Thresholds for aneuploidy were set at ζ>+3.0 and ζ<-3.0 for over- or under-representation, respectively, of a chromosome.

All aneuploidies yielded ζ-scores >3.0 for the aberrant chromosomes (range +3.38 to +9.43), whereas all other chromosomes were detected as normal (range -2.27 to +2.31). Our data thus indicate that full-blown fetal aneuploidies can be reliably detected in maternal plasma using a multiplex massively parallel sequencing approach. Further improvements in the rate of multiplexing samples to reduce costs and increase efficiency are currently under investigation.

C07.4*

The Use Of Array CGH for Prenatal Diagnosis Of Fetuses With Congenital Malformations Detected By Ultrasound

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Karyotyping has remained the gold standard for the detection of numerical and structural chromosome rearrangements for prenatal genetic diagnosis. Molecular karyotyping by array CGH is now a widely used technique for postnatal genetic analysis providing genome-wide detection of genomic imbalances at a resolution far superior to conventional karyotyping. The use of array CGH is an attractive alternative in the prenatal diagnostic setting, given the ability to overcome the resolution & time limitations of conventional karyotyping or the locus-specific limitations of targeted analyses such as FISH & MLPA.

We have undertaken a prospective study into the use of a high resolution array for the prenatal diagnosis of pregnancies where fetal abnormalities were detected on ultrasound scan. Over 100 fetal samples have been analysed from amniotic fluid and chorionic villi, using both direct and cultured material. Since UZ Leuven is a specialist referral centre for fetuses with isolated congenital diaphragmatic hernia (CDH), a subset of our referrals are from this cohort.

We show that array CGH detects a number of causal submicroscopic imbalances in fetuses with ultrasound anomalies. Of particular interest, our results allow us to refine the minimal critical region associated with CDH on chromosome 15q26 to a single gene, NR2F2 (COUP-TFII). We demonstrate an average reporting time of 8 days for uncultured material. We shall present our results, and also discuss our approach to the interpretation and reporting of CNVs detected prenatally.

C07.5

Diagnostic utility of array-based comparative genomicv hybridization (aCGH) in a prenatal setting

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Objective: Array-based comparative genomic hybridization (aCGH) is a technique for detecting submicroscopic deletions and duplications. There is limited information regarding its use in the prenatal setting. Here, we present our experience of 431 prenatal aCGHs between 2006 and 2011.

Materials and Methods: We reviewed the medical files of 431 prenatal cases seen in the Recanati Genetic Institute between 2006 and 2011 on whom prenatal aCGH was carried out.

Results: The indications for testing were fetal anomalies on ultrasound (U/S) (43%), advanced maternal age (AMA)(20%), family history of a disorder of unknown etiology (15%), parental concern (13%), Abnormal routine karyotype (8%) and abnormal serum biochemical screening for common fetal aneuploidies (1%).

Of 34 cases with a known abnormal karyotype, 26 (75%) had a normal aCGH. This enabled us to reassure the families and the pregnancies were continued. The remaining 8 (25%) showed an abnormal aCGH, confirming the chromosomes were unbalanced, and were terminated. Of 397 cases with a normal karyotype, 7 had an abnormal aCGH and after genetic counseling they were terminated. Overall, new clinically relevant results were detected by aCGH in 41 cases, providing additional information for prenatal genetic counseling and risk assessment.**Conclusion:** Our results suggest that prenatal aCGH should be offered particularly in cases with abnormal U/S (detection rate was 1:46). We found the rate of detecting an abnormality by aCGH in low-risk pregnancies (normal fetal U/S) was 1:70, but larger studies will be needed to expand our knowledge and validate our conclusions.

C07.6

Prenatal diagnostic testing of the Noonan syndrome genes in fetuses with abnormal ultrasound findings

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Noonan syndrome is a genetically heterogeneous, autosomal dominant disorder characterized by short stature, congenital heart defects, distinctive facial features, and a variable degree of intellectual deficits. The *PTPN11* gene is involved in 50% of Noonan syndrome cases, but is also found to be mutated in 9% of fetuses with cystic hygroma and increased nuchal translucency (NT) (Lee *et al.* (Clin Genet 2009;75:190)). In our laboratory parallel testing of *PTPN11*and *KRAS* in fetuses with a normal karyotype and an increased NT showed a mutation frequency of 16% [Houweling *et al*. (2010) Prenat Diagn 30:284]. Until now, we have investigated the DNA of 76 fetuses for mutations in *PTPN11*, *KRAS*, *SOS1,* and *RAF1*. In 12 fetuses (15,8%) a *de novo* mutation has been identified in either *PTPN11* (n=8), *RAF1* (n=3), or *KRAS* (n=1).

In an anonymous study the DNA of 62 other fetuses with increased NT (>3.5 mm), cystic hygroma, hydrops fetalis or congenital heart disease - with or without ventricular megaly and/or renal abnormalities- and a normal karyotype, has been investigated for mutations in 10 genes of the RAS-MAPK pathway. Besides potentially causative mutations in *PTPN11* (n=1), *RAF1* (n-1), and *SOS1* (n=1), also unclassified variants in *BRAF* (n=1) and *MAP2K1* (n=1) have been identified. We conclude that prenatal testing for fetuses with an increased NT (>3.5 mm) should at least include *PTPN11*, *RAF1* and *KRAS* sequencing. If enough time and DNA is left, mutation analysis of *SOS1*, *BRAF* and *MAP2K1* should be considered.

C08.1*

A *de novo* paradigm for mental retardation

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Recent studies have indicated that humans have an exceptionally high per-generation mutation rate of 7.6 x 10^{-9} to 2.2 x 10^{-8}. These spontaneous germ line mutations can have serious phenotypic consequences when affecting functionally relevant bases in the genome. In fact, their occurrence may explain why diseases with a severely reduced fecundity remain frequent in the human population, especially when the mutational target is large and comprised of many genes. This would explain a major paradox in the evolutionary genetic theory of cognitive disorders.

In this presentation, we will first describe our recent work on using a family-based exome sequencing approach to test this *de novo* mutation hypothesis in 10 patients with unexplained mental retardation[1]. Unique non-synonymous mutations were identified and validated in nine genes. Six of these, identified in different patients, were likely pathogenic based on gene function, evolutionary conservation and mutation impact. These findings provided a strong experimental support for a *de novo* paradigm for mental retardation.

The clinical relevance and ultimate proof for disease-causality of these novel genes lies in the identification of *de novo* mutations in additional patients of similar phenotype. As such, we are currently screening ~1,200 patients with unexplained mental retardation for mutations in *YY1*, representing one of these newly identified genes. Moreover, we are extending our family-based exome sequencing approach to 100 patients to establish the diagnostic yield for *de novo* mutations in patients with unexplained mental retardation.

[1]Vissers *et al*. A *de novo* paradigm for mental retardation. *Nature Genetics* **42**: 1109-1112 (2010).

C08.2

Homozygosity mapping, exon enrichment and next generation sequencing reveals single plausible gene defects in 72 consanguineous families with autosomal recessive intellectual disability

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Functional considerations and empirical data from animal models suggest that most gene defects are inherited as recessive traits. However, research into recessive disorders has lagged behind because in Western societies with their small-size families, most affected patients are isolated cases. Intellectual disability (ID) is a particularly important problem of health care world-wide, and most severe forms have a genetic etiology. Autosomal recessive forms of ID (ARID) are thought to be more frequent than X-linked ones, particularly in developing countries where parental consanguinity is common and the incidence of ID is significantly elevated.

Here we report on the first systematic attempt to shed more light on the molecular causes of ARID. By performing homozygosity mapping, exon enrichment and next generation sequencing (NGS), we have identified apparently causative gene defects in 72 out of 130 consanguineous families from Iran and elsewhere. In 26 families, causative changes were found in previously described disease genes. In three of these genes, i.e. AHI1, SRD5A3 and PRKCG, allelic mutations were found in more than one family. In 46 families, single apparently causative mutations were detected in genes not hitherto implicated in ID or related disorders such as autism, epilepsy or schizophrenia. Many of these genes are involved in (the regulation of) transcription and translation or other regulatory pathways known to be important for brain function, while others interact directly with known ID genes. Thus, the systematic identification of novel ID genes is also a potent strategy to unravel the pathways and pathogenetic mechanisms underlying cognitive disorders.

C08.3

Exome sequencing and ACAD9 mutation screening of patients with complex I deficiency

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Faulty energy supply due to defective oxidative phosphorylation is the biochemical signature of mitochondrial disorders, a genetically heterogenous group of rare, severe and highly invalidating human conditions. For a majority of patients, the molecular basis of the disorder remains unknown and there is an urgent need for new diagnostic strategies. We have recently used an exome sequencing strategy based on a single patient with a complex I deficiency to identify a pathogenic mutation in ADAD9, which codes for a member of the acyl-CoA dehydrogenase (ACAD) protein family. We had also shown that treatment of patient fibroblasts with riboflavin rescues the phenotype.

Here we demonstrate the efficacy of exome sequencing in combination with cellular assays in 12 additional unrelated complex I patients and the results of ACAD9 mutation screening in a group of 100 complex I patients. We found pathogenic variants in three known disease genes coding for mitochondrial proteins in four patients. In two instances, the pathogenic role of novel variants was established by the correction of the biochemical defect on expression of the wild-type protein in patient's fibroblasts. Fibroblasts of five patients with ACAD9 mutations were treated with riboflavin, the vitamin precursor of the FAD moiety, which is the catalytic cofactor of ACADs, is known to foster their assembly and stability. Riboflavin treatment of all five mutant cell lines resulted in a significant increase of complex I activity. This supports previous accounts of beneficial effects of a high dosage riboflavin regimen in ACAD9 patients.

C08.4

Next Generation diagnosis of Glycogen Storage Diseases

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Glycogen storage diseases (GSD) are heterogeneous disorders caused by mutations in one of 18 genes encoding proteins of the glycogen metabolism pathway. Clinical and genetic heterogeneity between GSD subtypes makes diagnosis lengthy and costly, requiring numerous clinical assessments including muscle or liver biopsy, followed by sequencing for mutations in a sequential 'gene-by-gene' manner in some cases. We have developed a rapid and cost effective Next Generation diagnostic test to screen all 18 genes simultaneously. A bespoke Agilent SureSelect in-solution DNA array is used to capture ~1Mb genomic sequence from all 284 exons of the GSD genes. The captured material is sequenced on the Illumina GAIIx platform with up to 12 samples per lane, using index tagging to reduce costs. The massive capacity of such instruments ensures adequate sequencing coverage (>30x) of target exons +/- 50bp, to accurately detect heterozygous variants. Validation experiments have correctly detected homozygous and heterozygous single nucleotide sequence variants, deletions ranging from 1bp to 38bp, a complex indel of 27bp, and large CNVs. We have tested 16 patients diagnosed clinically with GSD and have detected pathogenic mutations in 14, in the AGL, PHKA2, PHKB, PHKG2, PYGL, PYGM and SLC37A4 genes. Compared with the current costly and lengthy investigation of these patients, including sequential gene sequencing in some cases, this comprehensive mutation screen for GSD provides cheaper and faster diagnosis within a number of weeks rather than months or even years. This Next Generation diagnosis changes the patient's investigative pathway, reducing the need for alternative invasive diagnostic procedures.

C08.5

High Throughput Genetic Analysis in Patients with Hereditary Retinal and Optic Nerve Diseases

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Hereditary retinal dystrophies (RDs) and optic nerve degenerations (ONDs) are amongst the genetically most complex and heterogenous conditions. Up to now more than 180 genes have been described to be implicated in RDs and ONDs. However it has been estimated that mutations in the known genes account for only ~ 50% of all affected subjects. Current genetic diagnostics based on mutation scanning techniques (e.g. HRM, dHPLC, SSCP) and Sanger sequencing on capillary sequencing machines frequently takes several month or years and is laborious and cost-intensive. Such considerations, as well as the demand to clarify the remaining 50% of unsolved cases, have resulted in an initiative to transfer hereditary ophthalmic disease diagnostics to the high-throughput sequencing (HTS) arena.

Methods: DNA is enriched for the coding and flanking intronic/UTR sequences of a panel of 190 genes known to be associated with RD and OND diseases using a custom designed Agilent SureSelect kit. Sequencing is performed using barcoded libraries on a single Oct on the SOLiD 4 platform. The basic data analysis is performed with Bioscope v1.2. All variants and putative deletions are re-sequenced by the gold standard Sanger sequencing or quantitative PCR, respectively.

Results: Here, we present the results of the high throughput analysis in 50 patients. We will demonstrate how so far genetically undiagnosed cases could be solved by our newly developed method. Most interestingly, we are able to detect different types of mutations including SNVs, as well as copy number variations ranging from small, large up to whole exon deletion.

C08.6
Multiplex Targeted High-Throughput Sequencing for mendelian cardiac disorders identified oligogenic inheritance

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Cardiomyopathies and arrhythmias are common, seemingly mendelian autosomal dominant cardiac disorders known as primary cause of sudden cardiac death in young adults. These diseases are characterized by a remarkable genetic heterogeneity, which makes it difficult to unravel the causative mutation in a diagnostic laboratory.
To circumvent these limitations, we explored targeted high-throughput sequencing of multiplexed indexed samples. Five patients suffering from different mendelian cardiac disorders were analysed. We designed a capture microarray with the total genomic length of 1 Mbp that includes all exons/splicing sites of 132 genes involved in cardiovascular mendelian disorders. In Patient 1 with arrythmogenic right ventricular dysplasia (ARVD) and a known PKP2 frameshift deletion, an additional novel nonsense variant was found in Sodium Channel V (SCN5A) gene (p.Lys991X). In Patient 2 with hypertrophic cardiomyopathy (HCM), a novel missense variant in Acid Alpha-Glucosidase (GAA) gene and a second novel missense variant in the desmoglein-2 (DSG2) gene were identified. In Patient 4 with long-QT syndrome (LQTS) two missense variants in two different channel genes (SCN5A and KCNQ) were found. Finally, in Patient 5 with LQTS, a novel missense variant in the Fibrillin (FBN1) gene (Marfan syndrome) was detected. We did not identify any pathogenic mutation in Patient 3 with HCM. To our knowledge, this is the first report of such common oligogenic inheritance in mendelian cardiac disorders. Multiplex targeted high-throughput resequencing holds considerable promises for molecular diagnosis of highly heterogeneous disorders in clinical practice and allows a better understanding of the complexity of mendelian disorders.

C09.1
Dystrophinopathies and X-inactivation pattern: about 26 symptomatic carriers at pediatric age

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Aim: to define molecular basis underlying different clinical phenotypes of symptomatic DMD carriers at pediatric age.
Methods: 26 cases of early symptomatic DMD carriers followed in the french neuromuscular network were investigated. We report findings concerning clinical presentation, muscular histological analysis and type of gene mutation, as well as X-chromosome inactivation (XCI) patterns using DNA extracted from peripheral blood or muscle.
Results: The initial symptoms were significant weakness (88%) or exercise intolerance (27%). Clinical severity varied from a Duchenne-like progression to a very mild Becker-like phenotype. Cardiac dysfunction was present in 19% of the cases. Cognitive impairment was worthy of notice as 27% of the carriers are concerned. The muscular analysis was always contributive revealing muscular dystrophy (83%), mosaic in immunostaining (81%) and dystrophin abnormalities in Western Blot analysis (84%). 73% had exonic deletions or duplications and 27% had point mutations. XCI pattern was biased in 62% of the cases.
Interpretation: We report the largest series of manifesting DMD carriers and show that exercise intolerance and cognitive impairment may reveal symptomatic DMD carriers. The complete histological and immunohistological study of the muscle is the key of the diagnosis leading to the dystrophin gene analysis. Our study shows also that cognitive impairment in symptomatic DMD carriers is associated with mutations in the distal part of the DMD gene. XCI study does not fully explain the mechanisms as well as the wide spectrum of clinical phenotype, though a clear correlation between the severity of the phenotype and inactivation bias was observed.

C09.2
Development of systemic antisense treatment in dystrophic mouse models for Duchenne Muscular Dystrophy

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Antisense-mediated reading frame restoration is a promising therapeutic approache for Duchenne muscular dystrophy (DMD). It uses antisense oligonucleotides (AONs) to induce exon skipping to reframe mutated dystrophin transcripts, allowing synthesis of internally deleted, partly functional dystrophins. Proof of concept has been obtained in cultured cells and the mdx mouse model and this approach is currently tested in clinical trials by Prosensa/GSK (GSK2402968 and PRO044) and AVI Biopharma (AVI-4658).
The 2'-O-methyl phosphorothioate (2OMePS) chemistry used in the Prosensa/GSK trials has favorable pharmacokinetic properties, as it binds serum proteins, preventing clearance by the kidney and increasing serum half life. Uptake by healthy muscle is low, but is up to 10-fold higher in dystrophic muscle in animal models.
Here, we tested the safety and efficacy of high dose (200 mg/kg/week) AON treatment for up to 6 months in mouse models with varying levels of severity: mdx mice (mild phenotype) and mdx mice with one utrophin allele (mdx +/-; intermediate phenotype). This was well tolerated. Notably, in the more severely affected mdx +/- mice the therapeutic effect was larger: exon skipping and dystrophin levels were higher, the creatine kinase levels were more decreased and rotarod running time was more increased. Preliminary results suggest that AON levels in the muscles of the more severely dystrophic mdx +/- are higher than in those in mdx mice, confirming the hypothesis that AON uptake is aided by the disease pathology.
These results are encouraging for long term trials in patients, recently initiated by Prosensa Therapeutics/GSK.

C09.3*
A guideline for *CHD7* analysis

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CHARGE syndrome is a multiple congenital malformation syndrome. The causative gene, *CHD7*, was identified in 2004 and is a large gene comprising 37 coding exons. Not surprisingly, patients with a *CHD7*-mutation have a more variable phenotype than patients diagnosed with CHARGE syndrome based on the currently used clinical criteria. This raises the question whether CHARGE syndrome is a clinical or molecular diagnosis and in which patients *CHD7*-analysis should be done.
We explored the phenotypic spectrum of our cohort of 280 patients with a *CHD7*-mutation. The phenotype was highly variable, but external ear anomalies, cranial nerve dysfunction, semicircular canal hypoplasia and psychomotor delay were almost always present. In comparison with clinically diagnosed CHARGE patients cleft lip and/or palate and genital hypoplasia occurred more frequently and congenital heart defects less frequently in patients with a CHD7-mutation. In 5-10% of patients with typical CHARGE syndrome no *CHD7*-mutation could be identified. In these patients CHARGE syndrome remains a clinical diagnosis. In contrast, 14-17% of the patients with a *CHD7*-mutation do not fulfil the clinical criteria for CHARGE syndrome and in these patients *CHD7*-analysis can be helpful to establish the diagnosis. Confirming the diagnosis is important for the clinical surveillance of the patients. Moreover, finding a *CHD7*-mutation enables counselling of recurrence risk and reproductive options.
We propose a guideline for *CHD7*-analysis and indicate in which patients imaging of the semicircular canals is helpful. This guideline can help clinicians in their diagnostic work-up of patients suspected of CHARGE syndrome.

C09.4
The new Ghent criteria: What do they change?

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The diagnosis of Marfan syndrome (MFS) is challenging, and international criteria have been proposed. New diagnostic criteria for Marfan syndrome were released in 2010, giving more weight to aortic root aneurysm and ectopia lentis. We aimed to compare the diagnosis reached by applying this new nosology vs. the 1996 Ghent nosology in a well-known series of 1,009 probands defined by the presence of an *FBN1* mutation. 83% of patients could be classified as MFS according to the new nosology as compared to 89% according to the 1996 Ghent criteria. For the remaining 17% of patients, insufficient criteria had been reported to make a diagnosis of MFS, and the patients would be classified as ectopia lentis syndrome (ELS), Myopia - mitral valve prolapse - Aortic root dilation - Skeletal findings - Striae syndrome (MASS), Mitral Valve Prolapse Syndrome (MVPS); or potential MFS in patients aged less than 20 years. The possibility has to be considered that these patients would go on to develop classic MFS with time. Although the number of patients for a given diagnosis differed only slightly, the new nosology led to a different diagnosis in 15% of cases: 10% of patients with a previous diagnosis of MFS were classified as ELS or MASS in the absence of aortic dilatation; conversely, 5% of patients could be reclassified as MFS according to the new nosology in the presence of aortic dilatation in his personal or familial history. Whatever is the diagnosis, aortic follow-up is mandatory in all patients.

C09.5*
Mutations in *SMAD3* in the TGF-β pathway cause a new syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis

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Aortic aneurysms represent an important cause of cardiovascular morbidity and mortality. Thoracic aortic aneurysms and dissections are a main feature of some connective tissue disorders, such as Marfan syndrome and Loeys-Dietz syndrome.

We delineated a new syndrome presenting with aneurysms, dissections and tortuosity throughout the arterial tree in association with mild craniofacial features and skeletal and cutaneous anomalies. In contrast with other aneurysm syndromes, most of these affected individuals presented with early-onset osteoarthritis. We therefore propose to refer to this disorder as aneurysms-osteoarthritis syndrome (AOS). We recently mapped the genetic locus to chromosome 15q22.2-24.2. Subsequently, we identified mutations in *SMAD3*. This gene encodes a member of the TGF-β pathway that is essential for TGF-β signal transmission. *SMAD3* mutations lead to increased aortic expression of several key players in the TGF-β pathway, including SMAD3. We identified seven different mutations in unrelated families with AO syndrome. We present extensive clinical and molecular data from these patients among which a large family with 29 affected individuals.

Molecular diagnosis will allow early and reliable identification of cases and relatives at risk for major cardiovascular complications. Our findings provide models to study the development of both arterial wall anomalies and osteoarthritis and endorse the TGF-β signaling pathway as the primary pharmacological target for the development of new treatment strategies for aortic aneurysms and osteoarthritis.

C09.6
Novel Ehlers-Danlos Syndrome caused by mutations in the *CHST14* gene - Expanding the Phenotype

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Ehlers-Danlos Syndrome (EDS) is a heterogenous group of disorders characterized by varying degrees of connective tissue hyperextensibility. In 2010 a new type of EDS was described in patients of Japanese and of Turkish descent presenting with craniofacial characteristics, contractures and joint and skin laxity. All patients had mutations in the *CHST14* gene coding for the enzyme dermatan 4-O-sulfotransferase. We present the detailed clinical findings in 2 sisters of Afghani descent born to consanguineous parents. The patients presented with multiple contractures, progressive joint and skin laxity and hemorrhagic diathesis following minor trauma. Prenatal evaluation of patient 2 identified possible Dandy-Walker variant, prominent amnion-chorionic separation, bilateral club feet and clenched hands. Platelet aggregometry and electron microscopy revealed normal function and ultrastructure. Cultured fibroblasts produced normal amounts of fibronectin and elastin but demonstrated lack of collagen III production, lack of extracellular deposition of collagen 1 fibers and a peculiar intracellular retention of Collagen I. Both patients were found to be homozygous for a novel missense mutation in the *CHST14* gene.

The identification of two additional patients with this disorder raises awareness of the pan-ethnicity of the condition and expands our knowledge regarding its clinical manifestations. Our studies show that the condition has to be considered in cases presenting prenatally with clenched fists and amnion-chrion separation, that the facial features are characteristic regardless of ethnicity and the bleeding diathesis in these patients are not the result of thrombocytopathy. We also expand the knowledge regarding the collagen abnormalities in this condition.

C10.1
Exome sequencing reveals mutations in the retromer protein VPS35 as cause for Parkinson´s disease

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Parkinson's disease is the second most common neurodegenerative disorder affecting 1%–2% of the population above the age of 60. It is characterized by degeneration of dopaminergic neurons in the nigrostriatal pathway and other monoaminergic cell groups in the brainstem leading to bradykinesia, resting tremor, muscular rigidity, and postural instability as well as non-motor symptoms. To identify rare causal variants in late-onset Parkinson's disease (PD), we investigated a family from Austria with 16 affected individuals by exome sequencing. We found a missense mutation, D620N, in the retromer protein VPS35 in all seven affected family members that are alive. By screening additional PD cases, we saw the same variant co-segregating with the disease in an autosomal dominant mode with high penetrance in two further families with five and ten affected members, respectively. Genotyping showed that the shared haplotype extends across 65 kilobases around VPS35. Screening the entire VPS35 coding sequence in additional 860 cases and 1014 controls revealed six further non-synonymous missense variants. Three were only present in cases, two only present in controls, and one present in cases and controls. D620N and a further variant detected in a PD case, R524W, were predicted to be damaging by sequence-based and molecular dynamics analysis. VPS35 is a component of the retromer complex which mediates retrograde transport between endosomes and the trans-Golgi network and which has recently been described to be involved in Alzheimer's disease.

C10.2*

Fine mapping of the celiac disease locus on 3q27-q28 harboring the LPP gene

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Results from meta-analysis in genome-wide association studies (GWAS) revealed 26 loci to be associated with celiac disease, a common immune-mediated disease. The majority of the celiac loci also overlapped with results form GWAS conducted in other immune-mediated diseases, suggesting a shared genetic origin. One of the most strongly associated loci was found on 3q27-28 and this regions also seemed rather unique for celiac disease (rs1464510, p= 2.98 x 10^{-40}, OR=1.29). The region of association (18955-18960 Mb) is confined to within the LPP gene but to refine the region of association even further and to identify the haplotypes driving the association, we set out to fine-map the LPP region using different celiac populations, Dutch (803 cases and 846 controls), Finnish (647 cases and 1829 controls) and Italians (497 cases and 543 controls). We used genotype data from the celiac disease GWAS to look for others SNPs associated in this region in each population and also checked their linkage disequilibrium (LD) with rs1464510. We found different SNPs in each population with suggestive association and LD in Dutch (rs1559810, p=1.2 x 10^{-2}, r^2=0.8, D'= 0.9), Italian (rs6790359, p=7.8 x 10^{-4}, r^2 = 0.2, D'=0.9) and Finnish (rs9851967 p=2.1x10^{-4}, r^2=0.6, D'=0.8). Future analysis will focus on an imputated dataset, using IMPUTE 2 with HapMap III and 1000 Genomes Project as reference panels. To help to identify rare variants we plan to use additional European cohorts with high-density genotyping across the LPP region, including Spanish, UK, Pollish, and two non- European cohorts (Saharawi and Indian).

C10.3

A genome-wide association study identifies 2 loci associated with heart failure due to dilated cardiomyopathy

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In this first genome-wide association study of heart failure due to dilated cardiomyopathy (DCM) with discovery and replication cohorts comprising overall 2344 cases and 2410 controls we identified two DCM-associated SNPs, rs10927875 and rs2234962 with respective P-values of 9.5 x 10-10 and 4.0 x 10-12 in the combined data set. The first SNP is located at a locus on 1p36.13 which exhibit a yin/yang haplotype structure encompassing several genes including HSPB7. The second SNP on 10q26.11 is located within BAG3 and is non-synonymous. Sequencing of BAG3 exons in patients with familial DCM identified several damaging mutations which were absent in healthy individuals, suggesting that they are causal for familial DCM. HSPB7 and BAG3 encode for non-structural proteins important for autophagy, which implicates perturbed protein processing as a new mechanism underlying DCM-associated heart failure.

C10.4*

Accumulation of common lipid variants influences atherosclerosis, and incident cardiovascular disease

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A recent genome-wide meta-analysis of >100,000 individuals identified multiple loci associated with LDL-C, HDL-C and TG. Risk scores computed from the top SNPs in those loci successfully discriminated low-lipid controls from individuals with extreme lipid values. These same scores were used to test the hypothesis that the cumulative effects of common SNPs are associated with sub-clinical atherosclerosis and incident coronary heart disease (CHD) in the Rotterdam Study (n=8131) and a Dutch family-based cohort, the Erasmus Rucphen Family Study (n=2081). Both included measurements of carotid intima media thickness (IMT) and plaques. The Rotterdam Study additionally included information on incident CHD (mean follow-up time≈11 years). Controlled for age and sex, the LDL-C risk score was associated with IMT (*P*=0.021) and strongly associated with plaque (*P*=1.7x10^{-8}). Cox proportional hazards models were used to assess MI and CHD incidence. For each standard deviation increase in the LDL-C score, the hazard ratio (HR[95%C.I.]) for MI was 1.14[1.04,1.25] (*P*=0.006) compared to the mean score. For CHD, the per SD increase in risk was HR[95%C.I.]=1.10[1.04,1.50] (*P*=0.003). Exclusion of prevalent cases and control for additional covariates minimally affected the point estimates. The HDL-C risk score was nominally associated with plaque (*P*=0.049). We found no associations with the TG risk score and clinical outcomes. In conclusion, an accumulation of common genetic variants with small effects on lipid levels, particularly LDL-C, can significantly impact sub-clinical and clinical outcomes. As our knowledge of genetic variation increases, pre-clinical genetic screening tools might enhance the prediction and prevention of clinical events.

C10.5

Lipidomic Profiles as Endophenotypes for Predicting Diabetes Progression in Mexican Americans

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Lipids are a highly diverse class of molecule with essential roles in cellular structure, signaling, and energy storage. Dysregulation of lipid metabolism can lead to the development of various diseases including Alzheimer's, atherosclerosis, and diabetes. These lipid molecules may represent endophenotypes that are closer to gene action than classical lipid markers, and extremely valuable for genetic analysis. In a large-scale analysis of the human lipidome, we profiled 1,202 Mexican Americans from the San Antonio Family Heart Study, identifying and quantifying 356 different lipid species. We then performed quantitative genetic and genome-wide association analyses, using ~1,000,000 SNPs, to identify genetic factors influencing lipidomic profile variation. Most of the 356 lipid species are significantly heritable in our population. Results identified 128 lipid species that predict progression to diabetes in non-diabetics followed for ~10 years. The single best predictor of progression to diabetes is dhCer (dihydroceramide) 18:0, the biosynthetic precursor to ceramide 18:0. dhCer is significantly heritable (h^2=0.247; p=1.6×10^{-9}) and is markedly increased in diabetics (p=2.5×10^{-7}). In non-diabetics, those that progress to diabetes show higher dhCer 18:0 levels at baseline than non-progressors

($p=2.2\times10^{-8}$). This predictive relationship is maintained ($p = 1.2\times10^{-4}$) after accounting for baseline fasting glucose and insulin levels, indicating this lipid component may be an independent predictor of risk. A genome-wide association analysis reveals two rare SNPs on chromosome 3p22 (between *SNORA62* and *MOBP*) exhibiting significant association with dhCer 18:0 levels ($p=9\times10^{-8}$). Our results suggest that lipidomic profiles may represent endophenotypes that may help us identify genes involved in diabetes development.

C10.6*

Genetic association study of SNPs discovered from low coverage exome sequencing of 1000 phenotypically characterized Danish individuals

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Background and hypothesis: Genome-wide association studies (GWAS) have driven the identification of multiple new loci contributing significantly to modulation of the fasting plasma glucose levels in humans. However, the explained heritability is still very modest.

We hypothesize that novel exome variation not captured by GWAS may contribute to the missing heritability of fasting plasma glucose levels.

Methods: Exome capturing of 1000 Danish individuals were performed on the Nimblegen 2.1M followed by next generation resequencing with a mean depth of x8. Within a 34Mb targeted region we identified 70,182 SNPs with a MAF>1%.

Any association between the identified SNPs and fasting plasma glucose level was evaluated directly from the sequencing data, by accounting for the uncertainty of the genotype rather than on the called genotypes. The tests are based on score statistics which require less numerical optimization than conventional likelihood ratio test.

Results: We find many novel and known variants with allelefrequency down to 1% that associate with fasting plasma glucose levels at the significance level of<10-3. However, we were not able to replicate any of the top 20 variants by direct genotyping in independent study populations of 9,893 individuals.

Conclusion: Low-coverage exome sequencing driven association studies yield many novel low frequency-and common variants; however; it seems that there are no single low-frequency variants in the examined exome with a large effect on the fasting plasma glucose levels on its own, suggesting the need for testing various burden tests to evaluate the cumulative impact of multiple variants within the same locus.

C11.1

Mutations of *CEP152* encoding a centrosomal protein cause Seckel syndrome with defective DNA damage response

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Seckel syndrome is a rare autosomal recessive disorder characterized by intrauterine growth retardation, short stature, severe microcephaly associated with mental retardation, and a typical “bird-headed” facial appearance. Using a genome-wide SNP homozygosity mapping strategy we identified a homozygous donor splice-site mutation in *CEP152* cosegregation with the disease in six Turkish families. Independently, we identified *CEP152* as causative gene in an additional Seckel patient born to consanguineous parents using an exome sequencing strategy. Furthermore, additional screening identified two other patients carrying compound heterozygous mutations in *CEP152*. *CEP152* encodes a 1654 amino acid protein that was originally identified in a proteomic screen of human centrosomes. We could show that CEP152 deficient fibroblasts show severe defects in mitosis and spindle organization resulting in cells with multiple centrosoms and abnormal nuclei pattern. In addition, Seckel cells showed an overall increased sensitivity to oxidative stress and responded with increased apoptosis. Furthermore, DNA-damage response was impaired in cells with mutated CEP152 confirming a functional link between centrosomal function and DNA repair responses. We could show that impaired CEP152 function leads to genomic instability via increased H2AX phosphorylation due to enhanced activation of ATM signaling pathways. These findings provide evidence for an important role of CEP152 in DNA damage response and genome maintenance. In summary, we identified the centrosomal protein CEP152 as a novel protein involved in the maintenance of genomic integrity and in the ability to respond to DNA damage, and show that that recessive loss-of-function mutations in *CEP152* can cause Seckel syndrome in humans.

C11.2

NEK1 mutations cause short rib-polydactyly syndrome type Majewski

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Defects of ciliogenesis have been implicated in a wide range of human phenotypes and play a crucial role in different signal transduction pathways and cell cycle coordination. We thoroughly delineated the clinical, radiographic, and histological phenotype of the autosomal-recessive short-rib polydactyly syndrome Majewski type. Parametric multipoint linkage and haplotype structure analysis confirmed a linked interval with a maximum LOD score of 2.95 (17.36 Mb / 18.65 cM) on chromosome 4 encompassing 38 genes. Assuming an overlap of the phenotypic spectrum of our patients with other ciliopathies, we used known genes of the cilia proteome database and compared them with the genes in our candidate interval. The NIMA-related kinase 1 (*NEK1*) gene was highlighted as homozygous mutant mice show polycystic kidney disease, craniofacial anomalies, and growth reduction.

Sequencing of *NEK1* identified a homozygous nonsense and a

homozygous splice-site mutation in the affected persons of two consanguineous families as the underlying cause. *NEK1* encodes a serine/threonine kinase with proposed function in DNA double-strand repair, neuronal development, and coordination of cell cycle-associated ciliogenesis. With immunofluorescence analyses we confirmed that the absence of functional NEK1 significantly decreases the number of ciliated fibroblasts and alters the structural morphology of the primary cilium in vivo as well. Transmission electron microscopy indicated a defect in progression from stage I after vesicular accumulation of ciliogenesis to stage II and subsequent failure of axoneme growth. We further substantiate a proposed digenic diallelic inheritance of ciliopathies by identification of heterozygous mutations in *NEK1* and *DYNC2H1* in a further non-consanguineous family.

C11.3*

Whole Exome Sequencing Identifies The Genetic Cause Of A New Ciliopathy Syndrome

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Gene identification in rare single-gene disorders significantly lends to the understanding of key developmental and physiological processes. As 85% of all disease-causing mutations are within coding exons, the recent application of massive parallel sequencing with exon capture has shown the efficacy of this technique in the rapid identification of mutations in Mendelian disorders. Herein, the genetic basis of a novel syndrome was sought in a non-consanguineous kindred consisting of 6 offspring where 4 exhibited mid-gestation intrauterine death. Dysmorphic features included cleft palate, high nasal bridge, short columella, micrognathia, wide mouth and low set ears. Systemic features included renal hypoplasia, duodenal atresia and cerebellar vermis hypoplasia with hydrocephalus, pre and post-axial polydactyly consistent with a predicted ciliopathy. Linked regions were identified on 1p31.1, 4q,19p13.3 and 20q13.13 using high density SNP arrays (Affymetrix 250k/sty and Illumina 550K). As the identified regions were large, whole exome capture (Nimblegen EZ Exome SR, v1) followed by massively parallel sequencing (Illumina GAIIx) was employed. A novel compound heterozygous loss of function mutation involving an essential splice site and a truncating change were found in an annotated gene which segregated with affected family members. Subcellular localization revealed a nuclear, centrosomal and basal body localization for this protein. Gene knockdown in zebrafish results in a ciliopathic phenotype consisting of curved body axis, U-shaped somites, microcrania, microphthalmia, cardiac oedema and renal cysts. Whole exome sequencing has identified compound heterozygous mutations in a new ciliopathy gene and supports its role in gene identification in rare and novel syndromes.

C11.4

Costal2 (KIF7) mutations cause fetal Hydrolethalus and Acrocallosal syndromes and expand the ciliopathy spectrum

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KIF7, the human ortholog of *Costal 2*, represents a key component of the Hedgehog (Hh) signaling pathway. Here we report mutations in *KIF7* in patients with Hydrolethalus and Acrocallosal syndromes (HLS and ACLS), two multiple malformation disorders with overlapping features such as polydactyly, mid-line brain abnormalities, and cleft palate. Consistent with a role of KIF7 in Hh signaling, we show a deregulation of most of the Gli transcription factor targets and impaired GLI3 processing in *KIF7* mutated patient tissue, as well as defects in primary cilia length in fibroblast cells. *KIF7* is also a likely contributor of alleles across the ciliopathy phenotypic spectrum; sequencing of a diverse patient cohort revealed several missense mutations which, upon *in vivo* testing were shown to be detrimental to protein function. Consistent with an epistatic role for such alleles, *in vivo* genetic interaction studies indicated that *KIF7* could exacerbate the phenotype induced by the loss of other ciliopathy transcripts. Our data demonstrate the role of KIF7 in the Sonic Hedgehog signaling pathway in humans through the regulation of Gli targets, its role in primary cilia function and expand the clinical spectrum of ciliopathies.

C11.5*

Sensenbrenner syndrome is caused by dysfunctional IFT-A mediated retrograde transport in the cilium

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Sensenbrenner syndrome (a ciliopathy) is mainly characterized by skeletal and ectodermal anomalies, and can be accompanied by other features (e.g. chronic renal disease). We performed SNP array analysis and positional candidate gene sequence analysis in two siblings with Sensenbrenner syndrome of a consanguineous Moroccan family. Both patients suffered from nephronophthisis, and the youngest sibling had end-stage renal disease since the age of 3 years. We identified a homozygous mutation in the initiation codon of *C14ORF179* in both siblings. *C14ORF179* encodes IFT43, a subunit of the intraflagellar transport complex A (IFT-A) machinery of primary cilia. We show that the mutation disrupts translation; due to the mutation translation starts at an ATG in exon 2 instead of at the canonical initiation translation codon in exon 1. This results in a shortened N-terminally truncated IFT43 protein. The IFT-A protein complex (together with the dynein motor) drives retrograde axonemal transport in the cilium. We show that in fibroblasts of one of the affected siblings, disruption of IFT43 affects this transport from the tip of the cilium to its base. In fibroblasts of the patient with mutated *C14ORF179,* we found that IFT-B complex proteins aberrantly accumulate in the ciliary tips. Interestingly, we observed the same defect in fibroblasts from a Sensenbrenner syndrome patient with mutations in *WDR35/IFT121*, encoding another IFT-A subunit. Our results suggest that Sensenbrenner syndrome is caused by dysfunctional IFT-A mediated retrograde transport in the cilium.

C11.6*

CCDC39 is required for assembly of inner dynein arms and the dynein regulatory complex and for normal ciliary motility in humans and dogs

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Primary ciliary dyskinesia (PCD) is an inherited disorder characterized by recurrent infections of the upper and lower respiratory tract, reduced fertility in males and *situs inversus* in about 50% of affected individuals (Kartagener syndrome). It is caused by motility defects in the respiratory cilia that are responsible for airway clearance, the

flagella that propel sperm cells and the nodal monocilia that determine left-right asymmetry. Recessive mutations that cause PCD have been identified in genes encoding components of the outer dynein arms, radial spokes and cytoplasmic pre-assembly factors of axonemal dyneins, but these mutations account for only about 50% of cases of PCD. We exploited the unique properties of dog populations to positionally clone a new PCD gene, *CCDC39*. We found that loss-of-function mutations in the human ortholog underlie a substantial fraction of PCD cases with axonemal disorganization and abnormal ciliary beating. Functional analyses indicated that CCDC39 localizes to ciliary axonemes and is essential for assembly of inner dynein arms and the dynein regulatory complex.

C12.1

A genome wide study reveals rare CNVs exclusive to Alzheimer Disease extreme phenotypes

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Autosomal dominant early onset Alzheimer disease (ADEOAD) is a rare condition with a prevalence rate estimated to 5.3 per 100 000 persons at risk. Mutation screening of more than 150 ADEOAD families, ascertained in France by the National Centre for early-onset AD (CNR-MAJ) has shown that mutations of *APP*, *PSEN1* and *PSEN2* genes account for 85 % of ADEOAD families. We hypothesised that rare copy number variants (CNVs) could be involved in ADEOAD families without mutations in known genes. Using high resolution array CGH, we assessed the presence of rare copy number variants (CNVs) in two highly selected disease groups, 21 unrelated ADEOAD cases without alteration on *APP, PSEN1* and *PSEN2* genes and 12 isolated Alzheimer disease (AD) cases with age of onset before 55 years. This analysis revealed the presence of 7 singleton CNVs (4 in ADEOAD and 3 in isolated cases) not present in 1078 controls, thus yielding a nominal significance for association of every CNV with AD of p=0.019 in the ADEOAD group and p=0.011 in the isolated cases group, respectively. Strikingly, 4/7 rearrangements target genes encoded proteins that are tightly related to Amyloid β (Aβ) peptide metabolism or signalling. Although these variants are individually rare and restricted to particular subgroups of patients, their characteristics strongly support the causal role in human pathology of a set of genes coding for molecules suspected for a long time to modify Aβ metabolism or signalling and for which animal or cellular models had already been developed.

C12.2

Loss of Cav1.3 (CACNA1D) function in SANDD syndrome, a novel human channelopathy with bradycardia and congenital deafness

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Hearing impairment occurs in approximately 1 out of 500 newborns. Most cases with congenital deafness are of genetic origin. We identified a homozygous founder mutation in the alternatively spliced exon 8B of *CACNA1D*, the gene encoding the pore-forming αλπηα1-subunit of voltage-gated $Ca_v1.3$ L-type calcium (Ca^{2+}) channels, in two consanguineous deafness families. The mutation results in an insertion of a glycine residue in a highly conserved region near the channel pore. $Ca_v1.3$ channels tightly control Ca^{2+}-dependent glutamate release at cochlear inner hair cell (IHC) ribbon synapses in response to sound and the diastolic depolarization in sinoatrial node (SAN) pacemaker cells. Targeted deletion of $Ca_v1.3$ channels in mice causes deafness and pronounced, non-fatal SAN arrhythmia and bradycardia. Strikingly, all patients also had pronounced SAN dysfunction with SAN arrhythmia, bradycardia and junctional escape rhythms. We termed the condition SANDD syndrome (**s**ino**a**trial **n**ode **d**ysfunction and **d**eafness). We analyzed the biophysical properties of wildtype and mutant $Ca_v1.3$ channel complexes using whole-cell patch-clamp recordings. Homozygous individuals lack significant L-type Ca^{2+} currents from exon 8B-containing $Ca_v1.3$ channels. The SANDD phenotype and mouse mRNA expression studies suggest that the exon 8B splice variant is predominant in human IHC and SAN pacemaker cells. The *CACNA1D* mutation underlying SANDD results in non-conducting calcium channels and altered voltage-dependent gating. Our results constitute the first evidence that $Ca_v1.3$ channels are involved in human cardiac pacemaking, implying them as a potential drug target for controlling heart rate in disease conditions. Regular cardiological follow-up of SANDD patients seems appropriate to avoid cardiovascular complications.

C12.3

Validating massive parallel sequencing as a diagnostic tool for seizure disorders

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Introduction: The epilepsies are common neurological disorders with a strong genetic impact. Consequently, understanding the genetic basis of seizure disorders will provide novel insights into the underlying pathophysiology and result in novel diagnostic and therapeutic avenues. With our approach we aim to reveal the genetic basis of epileptic disorders in so far unresolved cases.
Methods: Genomic DNA is enriched for a panel of 485 genes using a custom designed Agilent SureSelect in solution kit. 265 of the 485 genes are known causative genes for seizure disorders comprising all relevant epilepsy phenotypes. The remaining genes on the panel represent putative candidate genes for epileptic disorders. Sequencing is performed on the SOLiD 4 platform. We developed a diagnostic pipeline to (i) identify regions that are underrepresented with reads, (ii) identify potentially pathogenic SNVs, (iii) identify small insertions and deletions and (iv) identify larger indels including whole exon deletions and duplications. All variants and putative deletions are then validated using conventional methods.
Results: We sequenced 20 so far genetically undiagnosed cases with a broad spectrum of epilepsy phenotypes. We present an overview of the number of detected sequence alterations comprising mutations, possibly damaging variants as well as benign SNPs in both well-known epilepsy genes and putative candidates.
Conclusion: We have successfully established a fast and cost efficient genetic screening method for patients with seizure disorders. By applying this approach we hope to uncover both known and unknown sequence variants and give new insights in genetic factors involved in epileptogenesis.

C12.4

The XLID protein PQBP1 is a novel regulator of RNA metabolism

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The polyglutamine binding protein 1 (PQBP1) gene plays an important role in X-linked intellectual disability (XLID). To date, about 25 families with a mutation in this gene have been identified. Common clinical features include intellectual disability, microcephaly, and short stature. Most of the mutations cause frameshifts, which result in premature stop codons. Interestingly the mutant mRNA is only partially degraded

via nonsense mediated mRNA decay and truncated protein exists (Musante et al., 2010).
To unravel the pathomechanism of the disease, we have searched for novel PQBP1 interactors. We have found several splicing factors, belonging to the SR protein family, which are important regulators of alternative splicing. One of the newly found PQBP1 interactors, SRp20, is highly expressed in brain. In RNA-protein immunoprecipitations, we could show that, in addition to SRp20 protein, the PQBP1 protein complex contains SRp20 mRNA. As other SR proteins, SRp20 is able to modulate its own protein level, by regulating alternative splicing of its RNA. To test whether PQBP1 is involved in this feedback mechanism, we have knocked-down PQBP1 and overexpressed a minigene containing the alternatively spliced exon of SRp20. RT-PCR experiments indicated that knock-down of PQBP1 significantly increased skipping of the alternatively spliced exon. Real time RT-PCR on endogenous SRp20 mRNA confirmed these results. Interestingly, rescue experiments performed with mutant PQBP1 constructs revealed deficient splicing activity. Confirmation of several PQBP1 regulated target RNAs are in progress. Taken together, we provide the first evidence that the XLID PQBP1 protein plays a key role in nuclear RNA metabolism.

C12.5*

Genetic cause for infantile mitochondrial cardiomyopathy identified by exome sequencing

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Mitochondrial dysfunction is an important cause of infantile cardiomyopathies, but most patients with the devastating, fatal disorder still remain without a molecular diagnosis. Here we investigated the molecular background of the disease in a patient who died at the age of 10 months of hypertrophic mitochondrial cardiomyopathy. The mitochondrial respiratory chain complexes were severely disturbed in the patient's heart and to a lesser extent in the skeletal muscle and brain. Since pathogenic mitochondrial DNA mutations were excluded as the cause of disease, we sequenced the whole-exome of the patient to reveal the underlying nuclear disease gene. Over 65,000 SNPs were identified and filtered using our on-site-pipeline. The data analysis led to a single gene encoding a novel mitochondrial protein, which was found to contain a homozygous missense mutation. Compound heterozygous mutations in the same gene were identified in a second family, with two affected children, who died of antenatal/neonatal hypertrophic cardiomyopathy. Protein modeling predicted different functional consequences of the two mutations for the enzyme activity, offering an explanation for the neonatal vs infantile disease onset in the patients. Our example shows that disease mutations of single patients can be identified by whole-exome sequencing. Consequently, the genetic background of pediatric mitochondrial cardiomyopathies is now starting to unravel. In conclusion, we have identified a new genetic cause of infantile cardiomyopathy, which may also underlie unexplained antenatal death.

C12.6

A novel GPI-deficiency related disorder: multiple congenital anomalies-hypotonia-seizures syndrome is caused by a mutation in *PIGN*

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Background. There are currently three human diseases known to be caused by mutations in proteins involved in GPI synthesis. We report on a hitherto undescribed GPI-deficiency related disorder. This autosomal recessive syndrome is characterised by dysmorphic features and multiple congenital anomalies together with severe neurological impairment, chorea and seizures leading to early death. Methods. Homozygosity mapping was performed using Affymetrix Human Mapping 250k NspI arrays. Sequencing of all coding exons of the candidate genes was performed with primer sets designed using the Primer3 program. Fluorescence Activated Cell Sorting was performed using conjugated antibody to CD59. Staining, acquisition and analysis was performed on a FACSCalibur flow cytometer.
Results. We mapped the disease locus to 18q21.32-18q22.1 and identified the disease-causing mutation, c.2126G>A (p.Arg709Gln), in *PIGN*, which encodes glycosylphosphatidylinositol (GPI) ethanolamine phosphate transferase 1, a protein involved in GPI-anchor biosynthesis. Arginine at the position 709 is a highly evolutionarily conserved residue located in the PigN domain. The expression of GPI-linked protein CD59 on fibroblasts from patients as compared to that in a control individual showed a ten-fold reduction in expression, confirming the pathogenic consequences of the mutation on GPI-dependent protein expression. Conclusions. The abundant expression of *PIGN* in various tissues is compatible with the diverse phenotypic features observed in the patients and with the involvement of multiple body systems. The presence of developmental delay, hypotonia and epilepsy combined with multiple congenital anomalies, especially ano-rectal anomalies, should lead a clinician to suspect a GPI deficiency-related disorder.

C13.1*

Copy Number Variants in a Large Pedigree of Inbred Mice

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Copy number variation (CNV) is an important source of genomic diversity that plays a role in disease, adaptation and evolution. Recent reports suggested the existence of hotspots favouring the appearance of CNVs in germline and somatic tissues, however the mutation rate of these variants remains largely unspecified. Rates of 3.6x10-3 to 1.1x10-2 have been estimated from 10 CNVs of 14 sub-populations of the C57BL/6J strain. Similarly, human parent-offspring trios allowed estimating a rate of 3x10-2 for a broader CNV size range. To assess how CNV alleles are transmitted from one generation to the next and estimate their mutation rate, we setup a large brother-sister mating of C57BL/6J mice over nine generations, for a total of 692 mice. We used high-resolution CGH arrays (2.1M features, mean probe spacing 1.2kb) to detect CNVs in 45 representative individuals from this cohort. We combined three independent detection methods, a Bayesian HMM, GADA and Nexus5.1, to identify 485 CNV regions (CNVRs) covering 24Mb (1%) of the mouse genome. We selected 185 high-confidence CNVRs and determined their absolute copy number with nanoString nCounter in the complete pedigree. To infer CNV alleles from absolute copy number for each individual, we devised a semi-automated strategy relying on trio information. We followed the inheritance of existing CNVs and identified de novo CNVs, some of which appeared multiple times independently in different branches of the pedigree. This allowed calculating a genome-wide mutation rate and ascertain general or gender-specific segregation bias of a large number of CNVs in a large pedigree.

C13.2

Balanced translocation and copy number detection in hematologic disorders by array CGH

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Microarray-based comparative genomic hybridization (aCGH) can detect submicroscopic copy-number alterations (CNAs) throughout the genome, but the technique cannot detect balanced translocations, which are important markers for hematologic disease. We report the development of a microarray-based technology coupled with linear DNA amplification, termed translocation-CGH (tCGH), that can identify and characterize balanced translocations in addition to CNAs. With this technology, we have developed tCGH assays for eight different hematologic disorders that simultaneously test for the presence of multiple, clinically relevant balanced translocations associated with each disease. Our data demonstrate the utility of the assay with the detection of >20 different balanced translocations in a variety of disease specimens. The ability of this technology to detect

clinically relevant balanced translocations, to identify specific partner chromosomes precisely, to delineate the molecular breakpoints within the single genes involved and to reveal submicroscopic CNAs at these breakpoints provides distinct advantages over the current standard of chromosome and fluorescence *in situ* hybridization (FISH) analysis. In addition, this technology is of value when cytogenetics or FISH fails, is ambiguous, or is discordant. The novel findings revealed by tCGH will have a profound impact on future algorithms used in the diagnosis, prognosis and treatment of individuals with hematologic and other malignancies.

C13.3
A standardized statistical decision procedure for mosaic detection by array CGH.

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Somatic chromosomal mosaicism is a well established cause for mental retardation and multiple congenital abnormalities (MR/MCA). In 0.4 - 1% of patients with MR/MCA a mosaic pattern is detected (Hoang et al, 2010). It has been reported that array-CGH is able to detect up to 5% mosaicism (Conlin et al, 2010). However, until now, detection of mosaicism is predominantly based on visual inspection of the graphical representation of the array results.

We developed a statistical decision procedure to detect mosaic genotypes. Input parameters are the assumed a priori probability, e.g. the chance of 0.0001% that at a certain chromosomal region mosaicism is present, the variability of probe intensities, the desired area under the curve (AUC) of the receiver operating characteristic (ROC) curve and the mean deviation of a specific interval. The output is the minimal number of adjacent probes needed for a significant deviation of the interval from the mean array intensity. For practical use we constructed tables. If an observed number of probes in an interval with a known average ratio surpass the threshold number of probes needed for the specific interval ratio given in the table, mosaicism is a reasonable explanation and confirmation studies should be performed. We aim to evaluate this decision procedure in a retrospective approach using 2000 patients with MR/MCA including 14 confirmed mosaics detected by visual inspection of the graph. The results of this retrospective study will be presented as well as a practical guideline for the use of the table in daily practice.

C13.4
A significant proportion of private inherited CNVs has pathogenic relevance in intellectual disability: clinical-genetic survey in 40 families.

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During routinary array-CGH analysis applied to the diagnosis of intellectual disability, private CNVs inherited from an apparently healthy parent represent an emerging category of chromosome imbalances with no clear-cut significance. It was recently suggested that rare inherited CNVs have to be considered benign variants.

We analyzed by a-CGH a total of 756 patients with intellectual disability usually associated with multiple phenotypical anomalies. Chromosomes imbalances were detected in 230 of them (30%). We classified the observed CNVs in the following categories: 1) clearly pathogenic, 132/756 (17.5 %); 2) likely benign: 58/756 (7.7 %); 3) with uncertain significance: 40/756 (5.3 %). This last category included private CNVs inherited from an apparently healthy parent, but not reported in Database of Genomic Variants. Each private inherited CNV was evaluated for gene content, size and nature of the rearrangement (deletion vs duplication), parental origin and the associated clinical phenotype. New and more detailed information about clinical history of the family were collected in each case. To speculate whether the observed CNV could act as pathogenic factor in intellectual disability, we looked at the following mechanisms: 1) mosaic status in unaffected parents (FISH on metaphase chromosomes or interphase nuclei) 2) reduced expressivity; 3) imprinting effects; 4) uncoverage of a recessive allele.

A total of 18 of 40 private inherited CNVs (44%), resulted to have pathogenic relevance. Specificity of both the genetic and clinical profile, and mechanisms, are discussed.

C13.5*
The power of high resolution non-targeted array-CGH in identifying intragenic rearrangements responsible for Cohen syndrome

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Cohen syndrome (CS) is a rare autosomal recessive inherited disorder that results from mutations of the *VPS13B* gene. It has been recently showed that copy number variation (CNVs) at *VPS13B* locus could result in CS when present in a compound heterozygous state associated to a point mutation or in a homozygous state. We screened for the *VPS13B* gene 54 patients from 46 families with CS and identified at least one mutation in 12 families, 6 of which had mutations in compound heterozygous state, 1 in a homozygous state and 5 had only one mutation. 244K array-CGH was performed in 2 groups, group 1 including the 5 families with one mutation and group 2 including families with no mutation but fulfilling the Chandler and Kolehmainen's criteria, to seek to identify a large intragenic event. We identified a CNV in 3/5 families from group 1 and in 1/9 families from group 2. A targeted 8x15K array-CGH was used in the 2 groups to look for a smaller intragenic rearrangement not detected by the 244K array-CGH but did not reveal any additional rearrangement. When pooling the different series, we found that a CNV could be detected in 33% of families with CS and showed that all of them could be detected by 244K array-CGH. These results confirm that CNVs are a major cause of CS and allowed us to suggest that non-targeted 244K array-CGH could be easily used as an initial screening method for the molecular diagnosis of CS.

C13.6*
Nasal speech and hypothyroidism are common hallmarks of 12q15 microdeletions.

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Array CGH has led to the identification of several microdeletion syndromes by screening individuals with shared phenotypical characteristics. By combining array CGH data from large cohorts of patients, the screening of overlapping microdeletions is made possible, which in turn can lead to the identification of shared phenotypic characteristics. Here we present the clinical and molecular data of three previously unreported patients with submicroscopic overlapping deletions distal to the 12q14 microdeletion syndrome at chromosome bands 12q15q21.1. The deletions presented in this study vary from 2.5 Mb to 2.59 Mb in size with a 1.34 Mb common deleted region containing six RefSeq genes. To our knowledge, only seven patients have been reported with deletions in this region (Meinecke et al., 1987; Watson et al., 1989; Perez Sanchez et al., 2004; James et al., 2005; Tocyap et al., 2006;.Yamanishi et al., 2008; Schluth et al., 2008). The deletion breakpoints in the literature vary from 12q13 to 12q23 and are associated with growth retardation, developmental delay and dysmorphic features. The previously reported cases were detected with standard cytogenetic techniques, except for the patient of Tocyap et al. (2006) where localization of microsatellite markers was investigated and the patient from Schluth et al. (2008) which

was fine mapped using a 1 Mb resolution array. Although a variable clinical phenotype is present in all patients, the three patients reported here all present with developmental delay or learning disability, nasal speech and hypothyroidism. In this study we further elaborate on the genotype-phenotype correlation associated with this deletion.

C14.1*

Cutting edge technology and old-fashioned relationships: ethical reflections on incidental findings arising from whole genome and exome sequencing

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Incidental findings, a common issue in neurology and oncology, are a pressing ethical issue in next generation sequencing because of that technology's potential to deliver an enormous amount of often unsought for information of widely varying significance. This paper is based on a systematic literature review of ethical reflections on the issue of incidental findings, specifically those arising from whole genome and whole exome sequencing in clinical and research genetics. A number of electronic databases were searched using the term "incidental findings" and variations of (bio)ethics, (bio-) medical and research, and 25 articles were ultimately included for systematic review as being detailed ethical reflections on incidental findings in general or incidental findings in clinical and research genetics in particular. The ethical issues raised can be divided into two main clusters. One cluster revolves around the incidental findings themselves. This cluster includes ongoing debate on how to classify incidental findings, a classification that determines whether and how disclosure to the patient or research participant will proceed. A second cluster revolves around the relationships between the various parties involved: professionals, patients or research participants, their families, the wider society, and overseeing bodies such as Institutional Review Boards. Where the emphasis is placed in each relationship has a bearing on the disclosure process, and there is a notable tension between research and clinical practice. We conclude that, cutting edge technologies notwithstanding, personal relationships between medical professionals and patients or research participants are key in realising the full promise of whole genome and exome sequencing.

C14.2

Public attitudes towards genetic testing revisited. Comparing opinions between 2002 and 2010

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Background: Ten years after the Human Genome Project, medicine has yet to see any large part of the promised benefits, and experts have tempered their high expectations. Aim of this study is to compare public attitudes and expectations concerning genetic testing over the years (2002 vs. 2010).

Methods: Cross-sectional questionnaire survey of a consumer panel representative for the Dutch population. The questionnaire was sent to 1,385 members (>20 years). A similar panel was used in 2002.

Results: In 2010 and 2002, the response was 70% (978) and 63% (817), respectively. More respondents (64%) now believe that knowledge about the genetic background of disease will help people to live longer as compared to 2002 (43%). Respondents want genetic tests to be available (64% vs. 61% in 2010 and 2002, respectively). Respondents also think that genetic testing will become more normal in different situations (57% vs. 45%).

Expectations on negative aspects of testing changed over time. On the one hand, respondents think more often that a dichotomy will emerge between people with 'good' and 'bad' genes as a consequence of testing (38% vs. 22%). On the other hand, respondents seemed less concerned that genetic tests would become necessary for establishing the height of health insurance premiums (34% vs. 42%).

Conclusions: The results suggest that over the past ten years, expectations of benefits, potential use and impact on society of genetic testing have increased among the public, as have worries on inequity, while worries about genetic testing for health insurance have decreased.

C14.3

Survey of European clinical geneticists on awareness, experiences and attitudes towards direct-to-consumer genetic testing

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An increasing number of private companies are now offering direct-to-consumer (DTC) genetic testing services. The tests offered range from tests for single gene, highly penetrant disorders to susceptibility tests for genetic variants associated with common complex diseases or with particular non-health-related traits. The aim of this study was to collect information regarding the awareness, experiences and attitudes of European clinical geneticists about genetic tests and test interpretations sold directly to consumers. European clinical institutes where genetic counselling is offered to patients were contacted. One-hundred and thirty-one of the three-hundred eligible respondents (44%) answered a questionnaire. Eighty-six percent (110/128) of the clinical geneticists said they were aware that companies are advertising and selling genetic tests directly-to-consumers. Of the 44% (54/121) of the respondents who had been contacted by patients who underwent DTC testing, almost all respondents (98%, 47/48) did discuss test results with the patients. The following respondents somewhat or strongly agreed that DTC genetic tests should be legally banned for following tests: prenatal gender tests (69%, 77/112); genome scans (63%, 70/112), 54%, athletic performance (54%, 61/113), preconceptional carrier tests (53%, 59/112) and ancestry testing (27%, 30/113). The results indicate that most European clinical geneticists have only limited experience with patients who have accessed direct-to-consumer genetic testing, however, these physicians are entering into patient-physician interactions with patients when requested to do so.

C14.4

Familial Breast cancer: Is it time to move from a reactive to a proactive role?

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Introduction: Until recently there was little evidence base that interventions would affect life expectancy in familial breast cancer other than from theoretically based decision analysis in *BRCA1* or *BRCA2* mutation carriers. In 2004 the UK NICE guidelines for familial breast cancer on women unaffected with the disease determined that 'Health Care Professionals should not actively seek to identify women with a family history of breast cancer'. Since 2004 a wealth of evidence on the efficacy of MRI screening and risk reducing surgery has emerged including the first real impact on survival.

Methods: A total of 1197 GPs and 1223 surgeons completed a questionnaire on risk communication and management of familial breast cancer in four EU countries (UK, France, the Netherlands and Germany). One question addressed whether or not health care professionals should actively seek to identify women with a family history of breast cancer.

Outcome: Responses were overwhelmingly against current NICE guidance on identification of women at risk of familial breast cancer. Amongst GPs 74.8% in Germany up to 97% in France either disagreed or strongly disagreed with the non proactive approach. Amongst surgeons the disagreement ranged from 73.1 in Germany to 90.8% in France.

Conclusion: We have shown there are strong views against the current reactive approach to familial breast cancer amongst GPs and surgeons. We believe the time is right to become more proactive in identifying women at increased risk of breast cancer so that advice on

surveillance and possible genetics referral can be reviewed.

C14.5*

Risk predictions from direct-to-consumer personal genome testing: What do consumers really learn about common disease risk?

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Objective: Direct-to-consumer (DTC) companies predict risks of common complex diseases on the basis of genetic markers. Given the low number of markers involved and their small effect sizes, it is unclear whether high-risk groups can be identified. We investigated the risk distributions generated by two DTC companies for 8 diseases.
Methods: We simulated genotype data for 100,000 individuals based on published genotype frequencies. Predicted risks were obtained using the formulas and risk data provided by the companies.
Results: The table presents observed and trimmed ranges of predicted risks. The two companies used different formulas to calculate risks. One company predicted risks higher than 100% for 5 out of 8 diseases, which for AMD concerned 1 in 200 individuals. Observed ranges were smaller for the second company, except for Type 1 Diabetes. Predicted risks higher than 50% were frequently observed for company 1, but were exceptions for company 2. When predicted risks of company 1 were calculated using the formulas of company 2, observed ranges were substantially smaller.
Conclusion: Whether companies can currently predict high risks of disease predominantly depends on the method used for their calculation.

Condition	Company 1			Company 2		
	Average risk	Range	Trimmed range	Average risk	Range	Trimmed range
AMD	8	0.1-100	0.3-100	7	0.4-46.9	0.9-46.9
Atrial fibrillation	25	13.8-100	15.0-67.2	27.2	23.2-63.3	23.2-55.4
Celiac disease	1	0.0-56	0.1-21.7	0.1	0.0-5.1	0.0-2.9
Crohn"s disease	0.5	0.0-8.6	0.0-3.0	0.5	0.0-8.4	0.1-2.1
Heart attack	42	9.0-100	14.3-100	21.2	17.5-25.5	17.5-25.5
Prostate cancer	16	1.9-100	3.4-68.4	17.8	1.2-59.4	3.5-40.8
Type 1 Diabetes	0.4	0.0-10	0.0-2.7	1	0.1-41.4	0.1-13.7
Type 2 Diabetes	25	4.1-100	8.2-69.5	23.7	9.1-48.6	12.2-41.4

Values are percentages. AMD=Age-related Macular Degeneration; Trimmed range=0.5% outliers removed (two-sided)

C14.6*

Clinical, biochemical and molecular overview of the Ehlers-Danlos Syndrome, vascular type: the Ghent experience

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Mutations in *COL3A1*, encoding type III (pro)collagen, cause vascular Ehlers-Danlos syndrome (EDS), a life-threatening heritable connective tissue disorder characterized by thin translucent skin, arterial/intestinal/uterine rupture, easy bruising and characteristic facies.
We reviewed clinical/biochemical/molecular data of 211 probands fulfilling the Villefranche criteria for vascular EDS. All probands were subjected to biochemical and molecular collagen analysis. In 100/211 (47%) probands a causal *COL3A1* mutation was identified. Evaluation of this *COL3A1*-mutation-positive group revealed that 57% of the probands fulfilled the Geneclinics criteria. This result shows that while the Villefranche criteria lack sensitivity, the Geneclinics criteria are too strict. Importantly, biochemical analysis revealed an abnormal type III (pro)collagen pattern in all but 6 *COL3A1*-mutation-positive patients, three of whom harboured a *COL3A1*-null-allele. In the *COL3A1*-mutation-negative group, only five probands revealed an abnormal pattern for type III (pro)collagen and only four probands showed an abnormal pattern for type I (pro)collagen, with an underlying *COL1A1* mutation. In one *COL3A1*-mutation-negative proband a *TGFBR2* mutation was identified.
In addition, we performed collagen analysis in 60 probands with isolated carotid dissection, all with normal results, which suggests another underlying cause for this disorder Based on these observations, we designed new, age-specific clinical criteria for vascular EDS which are more specific than the Geneclinics criteria and more stringent than the Villefranche criteria. Re-evaluation of the 211 probands showed that 97% of the *COL3A1*-mutation-positive probands (versus 57% with Geneclinics criteria) fulfilled these adapted criteria. Additionally, these results confirm the biochemical collagen analysis as highly sensitive for the diagnosis of vascular EDS.

C15.1

Epigenetic regulation of learning & memory in a *Drosophila* model for Kleefstra syndrome

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The epigenetic modification of chromatin structure and its effect on complex neuronal processes like learning and memory is an emerging field in neuroscience. However, little is known about the "writers" of the neuronal epigenome and how they lay down the basis for proper cognition. Here, we have dissected the neuronal function of the *Drosophila* euchromatin histone methyltransferase (EHMT), a member of a conserved protein family that methylates histone 3 at lysine 9 (H3K9). We identify EHMT as a regulator of peripheral dendrite development, larval locomotor behavior, non-associative learning and courtship memory. The requirement for EHMT in memory was mapped to a subset of neurons in the adult brain and memory was restored by EHMT re-expression during adulthood, indicating that cognitive defects are reversible in EHMT mutants. To uncover the underlying molecular mechanisms, we generated genome-wide H3K9 dimethylation profiles using ChIP-seq technology. Loss of H3K9 dimethylation in EHMT mutants occurs at 5% of the euchromatic genome and is enriched at the 5' and 3' ends of distinct classes of genes that control neuronal and behavioural processes that are corrupted in EHMT mutants. This study identifies *Drosophila* EHMT as a key regulator of cognition that orchestrates an epigenetic program featuring classic learning and memory genes. Our findings are relevant to the pathophysiological mechanisms underlying Kleefstra Syndrome, a severe form of intellectual disability caused by mutations in human *EHMT1*, and have potential therapeutic implications. Our work thus provides novel insights into the epigenetic control of cognition in health and disease.

C15.2*

Mutations in the *NSUN2* and *ZNF526* genes cause autosomal recessive intellectual disability in Middle Eastern populations with elevated frequency

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So far no more than six hotspot loci for unspecific or non-syndromic autosomal recessive intellectual disability (NS-ARID) have been identified (Kuss et al. 2011, Human Genetics Vol.129, 141-148). In this study we now resolved the underlying gene defect of MRT 5 and report three deleterious mutations in *NSUN2*. This gene encodes a methyltransferase, which catalyzes the intron-dependent formation of 5-methylcytosine at C34 of tRNA-leu(CAA). Two of the observed changes were nonsense mutations (p.Q227X and p.Q372X), which

cause a complete loss of *NSUN2* transcripts in the patients. In the third family we found an intronic exchange of an adenosine for a guanine 11 nucleotides upstream of exon 6. This change causes exon 6 to be skipped during splicing and results in the loss of the main transcript. Hence all mutations lead to a loss of NSUN2 protein function in homozygous mutation carriers and thus in all likelihood cause the patient phenotype. In order to gain further evidence for an involvement of NSUN2 in cognitive functions, we studied fruit fly mutants that lack the NSUN2 ortholog. These experiments revealed a marked learning impairment in mutant flies, which clearly underscores the relevance of NSUN2 in higher brain functions.

Moreover we present mutation screening results for another hot spot locus (MRT11) in three Iranian families with several NS-ARID patients overlapping on Chr19q13.2-q13.31. Mutation screening by hybridization based enrichment of the exonic regions of all genes from the respective intervals and subsequent next-generation sequencing revealed two different single nucleotide changes in *ZNF526*. Mutations in other genes were not observed.ZNF526 encodes a brain expressed C2H2 zinc finger protein with DNA binding properties that is thought to be involved in gene regulation. Each mutation affects a functional domain of ZNF526 and both alter the protein conformation, causing a putative functional impairment as suggested by *in silico* protein modelling. A decrease in DNA affinity was confirmed by CHIP-seq and array-based gene expression studies showed specific changes in the expression patterns of patient lymphoblastoid cells, which could be recapitulated in ZNF526-deficient neuroblastoma cells. Functional annotation showed significant enrichment of the deregulated genes in pathways that play a role in protein synthesis, mitochondrial dysfunction, energy metabolism and gene regulation. Several zinc finger proteins were found to be involved in the pathogenesis of X-linked ID, but ZNF526 is the first to be implicated in NS-ARID. Our results show that both *NSUN2* and *ZNF526* belong to the still few genes known to carry NS-ID-causing mutations in independent families, which suggests that defects in either gene belong to the more common causes of NS-ARMR, at least in the Iranian population.

C15.3*

Mutations in genes encoding adaptor protein complex 4 (AP-4) subunits cause a clinically recognizable autosomal recessive syndrome.

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Autosomal recessive inheritance accounts for nearly 25% of intellectual disability (ID) but the extreme heterogeneity of these conditions markedly hampers gene identification. Using a combination of autozygosity mapping combined to Sanger sequencing of candidate gene or identity-by-descent filtering of exome sequence data, we identified three mutations in the genes encoding adaptor protein complex 4 subunits : a nonsense mutation within the AP4S1 gene (c.124C>T;p.R42X), a frameshift mutation within the AP4B1 gene (c.487-488insTAT;p.E163X) and a splice mutation within the AP4E1 gene (g.542+1_542+4delGTAA). All affected patients presented with severe ID, normal head circumference or mild microcephaly, hypotonia that progressed to spastic paraplegia with hyperreflexia and hypertonia, deformities of feet, and little muscle mass of the lower extremities. Finally, patients show an extreme shy and amicable character with stereotypic laughter.

Adaptor complexes (AP1-4) are evolutionarily conserved heterotetrameric complexes that mediate different types of vesicle formation and the selection of cargo molecules for inclusion into these vesicles. Interestingly, two mutations affecting the AP4M1 and AP4E1 genes (a splice donor site mutation in the AP4M1 gene and a 192kb-long deletion encompassing the 5' end of the AP4E1 gene) have been recently found to cause cerebral palsy associated with microcephaly and severe intellectual disability.

Combined to previous observations, these results support the hypothesis of a crucial role of AP4-mediated trafficking in brain development and functioning and demonstrate the existence of a clinically recognizable AP-4 deficiency syndrome.

C15.4*

A novel dynamic mutation in AFF3 associated with developmental delay

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Many types of dynamic mutations are cytogenetically expressed as fragile sites. Although it is known that fragile sites of the rare, folate sensitive type occur more frequently in cohorts of patients with cognitive disorders, few have been characterized at the molecular level. We studied three families with FRA2A expression. The probands were characterized by slow early motor and language development and learning disability. As the molecular basis of this fragile site we identified an elongated polymorphic CGG repeat in the 5' region of AFF3. This gene is a member of the family of nuclear transcription factors including AF4, AF5q31 and FMR2, involved in regulation of gene expression, cell expansion, embryonic development and fulfill a role in the central nervous system. Interestingly, AFF3 is a paralog of FMR2, causative of FRAXE non-syndromic mental retardation. Loss of expression of FMR2 occurs through dynamic repeat expansion of a CGG repeat in the 5' untranslated region of the gene and subsequent hypermethylation of the promoter. This mechanism was first described in the fragile X syndrome. Expansion of the AFF3 associated repeat was shown in the probands of the 3 families and in five family members using Southern blot analysis. By cSNP-analysis we could confirm that methylation of the promoter region silences transcription of the AFF3gene. All patients but not all parents were methylated. In conclusion we collected compelling evidence that transcriptional silencing of the AFF3 gene could play a role in the cognitive impairment present in all patients.

C15.5

Mutation in ARID1B as a frequent cause of intellectual disability

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Interstitial deletions of chr.6q are rare findings ranging from 0.3 to several megabases. Some associated features are common, including intellectual disability (ID), hypotonia and facial dysmorphism.

We detected a 2.3Mb *de novo* deletion in chr.6q25 with molecular karyotyping in an infant with ID, facial dysmorphism, strabismus and hyperopy.

This deletion lies within previously reported regions and encompasses only 5 known genes. We hypothesized that haploinsufficiency for one of these may impair normal development. To identify the phenocritical gene we screened these five positional candidates for point mutations in 331 patients with moderate to severe ID.

In one of these genes, ARID1B, highly expressed in brain and encoding a subunit of the mammalian SWI/SNF complexes important for different neuronal developmental programs, we found four *de novo* sequence alterations. One 11bp deletion resulting in a premature stop codon, which shortens the protein by 58AA, a stop mutation in exon 16 and an in-frame 3bp deletion. We thus identified a sequence alteration in ARID1B in nearly 1% of patients with unexplained intellectual disability. Additionally, CNV screening in 1,986 patients with intellectual disability revealed a validated *de novo* duplication of three exons resulting in a frame shift. All five patients display a pronounced clinical overlap like ID, especially regarding expressive speech and similar facial phenotype.

Moreover, recently the disruption of ARID1B was also reported in a translocation patient with ID, supporting our hypothesis of ARID1B mutation as a frequent cause of ID.

This study is part of the German Mental Retardation Network (www.german-mrnet.de).

C15.6*

Haploinsufficiency of microRNA-137 is associated with intellectual disability in patients with overlapping deletions in 1p21.3.

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Introduction: Genome-wide array technologies have led to the identification of several microdeletion and microduplication syndromes and genes involved in intellectual disability (ID). Microdeletions and/or duplications restricted to chromosomal region 1p21.3 have rarely been reported before. Several ID syndromes have been linked to the microRNA (miRNA) pathway, but there are no previous reports of genome wide array studies that have led to the identification of miRNAs associated with ID.

Methods: We characterized the clinical and molecular features of three sibs and 2 non-related patients with overlapping 1p21.3 deletions, detected by genome-wide array analysis. The deletions in the two unrelated patients were *de novo*. We determined the shortest region of overlap (SRO) to identify candidates for the phenotype.

Results: Clinical features comprised mild ID and autism spectrum disorder. The SRO was 1.22 Mb and included one annotated gene, *DPYD* (dihydropyrimidine dehydrogenase deficiency), which was unlikely to cause the phenotype, since no associated metabolic defect was detected. Interestingly, the SRO also comprised microRNA-137 (miR-137), which was shown to be under epigenetic regulation of *MeCP2* and involved in adult neurogenesis in previous studies in mice. Our patients had a significantly altered expression of miR-137 and downstream targets. Furthermore, in mouse synaptosomes we found significant enrichment of miR-137 at the synapse as assessed by qRT-PCR, suggesting a role for miR-137 as a modulator of local translation at the synapse.

Conclusion: We propose that these patients represent a novel microdeletion syndrome that is caused by haploinsufficiency of miR-137.

C16.1

High-resolution whole genome sequencing reveals specific chromatin domains from most human chromosomes associate with nucleoli

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TThe nuclear space is mostly occupied by chromosome territories and nuclear bodies. While the nuclear organisation of chromosomes affects gene function, relatively little is known about the role of nuclear bodies in the organization of chromosomal regions. The nucleolus is the best-studied subnuclear structure and forms around the ribosomal RNA repeat-gene clusters on the acrocentric chromosomes. In addition to rDNA, other chromatin sequences also surround the nucleolar surface and may even loop into the nucleolus. These additional nucleolar-associated regions have not been characterized.

We present here a whole-genome, high-resolution analysis of chromatin endogenously associated with a subnuclear body. We have used a combination of three complementary approaches, namely fluorescence-comparative-genome in situ hybridisation, high-throughput deep-DNA-sequencing and photoactivation combined with time-lapse fluorescence microscopy to study nucleolar-associated chromatin.

The data show that specific sequences from most human chromosomes associate with nucleoli in a reproducible and heritable fashion. The nucleolar-associated loci have in common a high density of AT-rich sequence elements, low gene density and a striking and significant enrichment in transcriptionally repressed genes. Unexpectedly, both the direct DNA sequencing and fluorescence photoactivation data show that certain chromatin loci can specifically associate with either the nucleolus or the nuclear envelope.

We are currently comparing nucleolar-associated loci in a model for cancer versus non-cancer cells. This will allow us to identify genomic regions that are mis-localized and of which gene expression maybe deregulated in cancer cells, and therefore can possibly be used as a probe to diagnose specific cancers or cancer stages.

C16.2*

Interrogation of high purity primary cell subsets demonstrates the majority of eQTLs operate in a cellular discrete manner

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A functional consequence of genetic variation is the regulation of transcript abundance - the formation of expression quantitative traits (eQTLs). In cultured umbilical-cord derived cells it is clear that many SNPs form eQTLs dependent upon the cell type. The degree to which these loci are reproducibly associated with gene expression in primary tissues and whether they act in a cell-specific manner is unclear. We have sought to identify polymorphisms acting in a cell specific manner from physiologically relevant tissue - freshly purified positively selected lymphoid derived CD19+ B-cells and myeloid derived CD14+ monocytes.

Cells were purified from a cohort of 288 healthy adult Caucasians. Subjects were genotyped at 731,442 markers (Illumina HumanOmniExpress) and expression analysed using whole genome microarrays (Illumina HumanHT-12, n=566). At a p-value <1x10-11 we identify 1960 SNPs with eQTLs unique to B-cells and 3700 SNPs with eQTLs unique to monocytes. Whilst the most significant eQTLs tend to be shared between cell type, over 90% of SNPs with eQTLs at a p-value <0.01 are cell specific. Furthermore, we identify a subset of ~20 genes with SNPs that lead to opposing directional effects on expression in a cell dependent manner. We confirm the microarray results for multiple genes using quantitative PCR and analyse several monocyte and B-cell specific eQTLs in a third cell type - Natural-Killer cells. Interrogation of this dataset suggests that several GWAS loci are associated with regulation of expression of genes in cis with the putative functional gene, indicating alternative candidate genes for several disease associations.

C16.3

Whole-exome sequencing of DNA from peripheral blood mononuclear cells (PBMC) and EBV-transformed lymphocytes from the same donor

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The creation of lymphoblastoid cell lines (LCLs) through Epstein-Barr virus transformation of B lymphocytes can result in a valuable and renewable biomaterial. Many biobanks, including the NINDS Human Genetics DNA and Cell Line Repository, establish and distribute LCLs and LCL-derived DNA samples. While LCLs have been extensively used in cellular and genetic studies, the process of cell immortalization and expansion during culturing may introduce genomic changes that could impact interpretation of genetic findings. We tested this hypothesis by performing whole exome-sequencing using both PBMC- and LCL-derived genomic DNA from a family of 4 individuals. Using the Agilent SureSelect Human All Exon Kit and SOLiD 4 Sequencer the DNA samples were sequenced to over 100X read depth with both DNA sources yielding similar sequencing efficiencies. An average of 17,730 SNPs were identified with 2% novel SNPs, as defined by no representation in dbSNP and/or the 1000 Genomes dataset. A comparison of sequence data from peripheral blood- and LCL-derived DNA from the same individual will be presented, including concordance rates, categorization of variant types and a breakdown

of novel variants by their potential impact. These findings will aid the research community in identifying the optimal starting material for deep sequencing studies.

C16.4
Unmasking of pathogenic rare variants by inherited deletions detected with multiplexed capture array-based re-sequencing.

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We investigated the hypothesis that a familial deletion, transmitted by a healthy parent to a child with mental retardation and congenital anomalies unmasks a recessive mutation transmitted by the other healthy parent. To detect such mutations, we designed two capture arrays with 60-mer probes tiled with an off-set of 10 nt covering the entire coding sequence (1.53 Mb capture footprint) of all genes within 20 transmitted deletion regions (covering in total 57.9 Mb genomic DNA). Deletions varied in size from 0.15 to 11.0 Mb and contained up to 36 protein coding genes. The patient DNA samples were sheared, ligated with short adaptors and SOLiD library barcodes, hybridized simultaneously (as pools of 9 and 11 samples, respectively) onto capture arrays, recovered and sequenced on a SOLiDv3.5 sequencer. Sequencing reads were mapped against the human reference genome and all Single Nucleotide Variations (SNVs) were compiled using in-house developed SNP-calling algorithms. Except for SNVs coinciding with indels and segmental duplications, all SNVs were close to 0 or 100% non-reference calls, confirming the mono-allelic nature of the regions studied. In all three cases tested, SNVs showed high concordance with SNP calls by Illumina HumanHap 300v3 BeadChips. Most of the 668 SNVs detected occurred in multiple samples. Only 9 SNVs located exclusively to the cognate deletion regions of the patients and were confirmed by capillary sequencing. Two patients carried a second, smaller loss within their inherited deletion region. All SNVs were evaluated for potential pathogenicity using inheritance patterns, Grantham matrix scores and evolutionary conservation.

C16.5
De novo copy number variations causing mental retardation show a significant paternal origin bias and are associated with increased paternal age

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De novo mutations and structural rearrangements are a common cause of mental retardation (MR) and other disorders with reduced or null reproductive fitness. Insight into the genomic and environmental factors predisposing to the generation of these *de novo* events therefore is of significant clinical importance. In this study we used information from SNP microarrays to determine the parent-of-origin of rare *de novo* CNVs from a large mental retardation cohort (3,443 patients, 227 *de novo* CNVs). The large majority of these CNVS originated on the paternal allele (78%, $p=3.4x10^{-9}$). This paternal bias was independent of CNV length and CNV type. Interestingly, the paternal bias was less pronounced for CNVs flanked by low-copy repeat (LCRs), suggesting that molecular mechanisms involved in the formation of rare *de novo* CNVs may be dependent on the parent-of-origin. In addition, a significantly increased paternal age was only observed for those CNVs which were not flanked by LCRs (p=0.009). This indicates that *de novo* CNVs are increasingly being generated with advanced paternal age by replication-based mechanisms during spermatogenesis.

C16.6*
Functional validation of GWAS loci for platelet traits in *Danio rerio* reveals new regulators of haematopoiesis

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A recent meta-analysis of the genome-wide association study (GWAS) in 68,000 individuals of European ancestry has identified 68 genomic loci (53 new) associated with platelet count and volume. Our functional study in *Danio rerio* by the means of gene silencing, has demonstrated a role for five genes from the associated loci (*ak3, arhgef3*, *jmjd1c*, *rnf145* and *tpma*) in regulation of thrombopoiesis. Additionally, lineage relatedness of megakaryocytes and erythrocytes prompted us to explore the putative role of these genes in erythropoiesis. Morpholino (MO) knockdown of all five genes but *tpma* resulted in a variable decrease in the number of erythrocytes. *Arhgef3*-depletion had the most profound effect on erythropoiesis, leading to further detailed studies. Examination of peripheral blood from *arhgef3* MO-injected embryos revealed microcytic, hypochromic erythrocytes, a classic feature of iron deficiency. Anaemia was rescued by intracellular supplementation of iron to *arhgef3* MO-injected embryos, demonstrating that *arhgef3*-depleted erythroid cells, once provided with intracellular iron are fully capable of haemoglobinisation. Disruption of the *arhgef3* target, RhoA, also produced severe anaemia, which was again rescued by iron injection. Moreover, silencing of *ARHGEF3* expression in the erythromyeloblastoid cell line K562 revealed that the uptake of iron transporter, transferrin, was severely impaired. This is the first study to provide evidence for ARHGEF3 being a regulator of transferrin and iron uptake in erythroid cells, through activation of RhoA.

In conclusion, our findings demonstrate the value of pursuing GWAS signals as a new and exciting forward genetics approach in identifying novel regulatory molecules and signalling pathways in haematopoiesis.

C17.1
Hypofunctional CHD1L variants in patients with congenital anomalies of the kidneys and urinary tract (CAKUT)

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Background. Recently, we identified a microduplication in chromosomal band 1q21.1 encompassing the *CHD1L/ALC1* gene encoding a chromatin remodeling enzyme in a CAKUT-patient.

Methods. To explore the role of *CHD1L* in CAKUT, we screened 85 CAKUT-patients for mutations in the *CHD1L* gene and performed functional analyses of the three heterozygous missense variants detected. In addition, we quantitatively determined *CHD1L* expression in multiple human fetal and adult tissues and analyzed expression of CHD1L protein in human embryonal, adult and hydronephrotic kidney sections.

Results. Two of three novel heterozygous missense variants identified in three patients were not found in more than 200 control chromosomes. All variants lead to amino acid substitutions in or near the CHD1L macro-domain, a poly-ADP-ribose(PAR) binding module interacting with PAR polymerase 1 (PARP1), and showed decreased interaction with PARP1 by pull-down assay of transfected cell lysates. Quantitative mRNA analysis demonstrated high *CHD1L* expression in human fetal kidneys, and levels were four-times higher than in adult kidney. In the human embryo at 7-11 weeks gestation, CHD1L immunolocalized in the early ureteric bud and the S- and comma-shaped bodies, critical stages of kidney development. In normal postnatal sections, CHD1L was expressed in the cytoplasm of tubular cells in all tubule segments. CHD1L expression appeared higher in the hydronephrotic kidney of one patient with a hypofunctional *CHD1L* variant than in normal kidneys, recapitulating high fetal levels.

Conclusions. Our data suggest that *CHD1L* plays a role in kidney development and that hypofunctional *CHD1L* variants may induce deficits in nephron differentiation leading to CAKUT.

C17.2*

First implication of STRA6 mutations in isolated anophthalmia, microphthalmia and coloboma

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Microphthalmia, anophthalmia and coloboma are structural congenital eye malformations that cause a significant percentage of childhood visual impairments. Several disease genes and risk loci have been identified but do not account for all MAC cases, suggesting that additional disease loci exist. Using SNP homozygosity mapping and targeted sequencing of the candidate homozygous segments, we identified a novel disease gene, *STRA6*, for isolated autosomal recessive colobomatous micro-anophthalmia (arCMIC). The *STRA6* missense mutation was subsequently detected in additional families that presented at the genetics clinic, including one patient with Matthew-Wood syndrome (MWS). We have shown that the mutant STRA6 protein is not expressed on the cell surface and has severely reduced vitamin A uptake activity. Furthermore, inhibition of retinoid synthesis in zebrafish embryos recapitulated the arCMIC phenotype, suggesting that diminished retinoid levels due to decreased vitamin A uptake by STRA6, account for the eye malformations in the arCMIC patients. We demonstrate that *STRA6* mutations can cause isolated arCMIC in addition to MWS, which opens the debate as to the genetic and environmental factors which determine whether patients with *STRA6* mutations develop isolated eye malformations or multisystem congenital anomalies. Currently, *STRA6* screening for diagnostic purposes is only offered to patients with MWS. Our findings suggest that patients with isolated eye defects of unknown etiology should also be considered for *STRA6* screening. The study also illustrates that the combination of homozygosity mapping together with targeted array-based sequencing is a powerful tool for identifying disease genes for recessive disorders.

C17.3*

Mutations in the 5' UTR of ANKRD26, the Ankirin Repeat Domain 26 Gene, cause an Autosomal-Dominant form of inherited Thrombocytopenia, THC2

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Thrombocytopenia 2 (THC2 [MIM 188000]) is one of the rarest forms of autosomal-dominant thrombocytopenia. Until recently, it has been reported in only two families. THC-2 affected individuals have a degree of thrombocytopenia ranging and platelets showing no relevant morphological abnormality. It had been mapped to chromosome 10p11.1-p12 and ascribed to mutations in MASTL or ACBD5. We showed that ANKRD26, another gene within the THC2 locus, and neither MASTL nor ACBD5, was mutated in eight unrelated families. ANKRD26 was also found to be mutated in the family previously reported to have an ACBD5 mutation. We identified 6 different ANKRD26 mutations, which were clustered in a highly conserved 19 bp sequence located in the 5' untranslated region. Mutations were not detected in 500 controls and are absent from the 1000 Genomes database. Available data from an animal model and Dr. Watson's genome give evidence against haploinsufficiency as the pathogenetic mechanism for ANKRD26-mediated thrombocytopenia. The luciferase reporter assay suggests that these 5' UTR mutations might enhance ANKRD26 expression. ANKRD26 is the ancestor of a family of primate-specific genes termed POTE, which have been recently identified as a family of proapoptotic proteins. Dysregulation of apoptosis might therefore be the pathogenetic mechanism, as demonstrated for another thrombocytopenia, THC4. Further investigation is needed to provide evidence supporting this hypothesis. (Am J Hum Genet. 2011 Jan 7;88(1):115-20)

C17.4

Genomic instability represents the unifying molecular pathology of progressive muscular dystrophies and muscle-derived sarcomas

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Muscular dystrophies (MDs) represent genetically heterogeneous disorders, characterized by progressive loss of functional muscle fibres and its replacement by adipose and fibrous tissue. Despite earlier promises based on the discovery of molecular defects underlying several MDs there is still no causative therapy, which underscores the need for a comprehensive understanding of the molecular mechanisms underlying the common MD pathology.

Investigating age-related phenotypes of MD mouse models, we observed the frequent occurrence of spontaneous skeletal muscle-derived tumors in mice lacking dystrophin, dysferlin, calpain-3, or Large, respectively. The primary gene defect and genetic background influenced tumor incidence, latency, localization, and gender prevalence. Combined loss of dystrophin and dysferlin or calpain-3 showed a strong synergistic effect. We found that these musculature-derived tumors are mixed sarcomas with rhabdomyo-, fibro-, and liposarcoma elements. Molecularly, these sarcomas shared non-random genomic alterations including frequent losses at the *Cdkn2a* and *Nf1* loci, amplification of oncogenes (*Met*, *Jun*), and recurrent duplications of whole chromosomes 8 and 15. Skeletal muscles of aged MD-mice without overt sarcomas regularly harbor sarcoma-specific genetic lesions. Consequently, we discovered that also dystrophic muscles and cultured cells from human MD patients are very similarly affected by DNA damage and chromosome copy number aberrations.

Thus, we present a novel, unifying concept for the molecular pathogenesis of MDs. Our identification of tumor-related DNA-pathologies in muscular dystrophies builds a bridge between a whole group of different rare diseases and the common entity of cancer diseases. This will foreseeably make the arsenal of anti-cancer therapies available for combating muscular dystrophies.

C17.5

A new human ichthyosis gene revealed by the canine genetic model

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Due to the unique history of dog breeds, the proximity between dog and human lifestyle, physiology and genetic diseases, dog constitutes a unique model to unravel the phenotype/genotype relationships and to provide new insights into human genetic diseases. In the golden

retriever breed, a high incidence of an autosomal recessive form of ichthyosis, resembling human autosomal recessive congenital ichthyoses (ARCI), has been diagnosed. Ichthyoses consist of a heterogeneous group of genodermatoses characterized by abnormal desquamation over the whole body, for which the genetic causes of several human forms remain unknown. We carried out a genome-wide association study on 40 unrelated golden retriever dogs (20 cases and 20 controls), using the canine 49,000 SNPs array (Affymetrix v2). The statistical analysis demonstrated a significant associated locus of 7 Mb on canine chromosome 12. Sequencing metabolic candidate genes revealed a recessive exonic indel mutation leading to a premature stop codon in one annotated gene. We then searched for alterations in the orthologous human gene in ARCI families, for which no genetic cause has been identified so far. We identified two recessive mutations in the catalytic domain of the orthologous human gene in two families. In addition to identify a seventh gene for human ARCI, electronic microscopy investigations highlighted the implication of this new gene product in the lipid metabolism of the skin barrier. Other breeds are affected by different ichthyoses, therefore using the canine model might facilitate the identification of rare gene variants responsible of human ichthyoses.

C17.6*

siRNA silencing of proteasome maturation protein (POMP) expression activates the unfolded protein response and constitutes a model for KLICK syndrome

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KLICK genodermatosis is an autosomal recessive skin disorder associated with a single-nucleotide deletion in the 5'untranslated region (UTR) of the proteasome maturation protein (*POMP*) gene. The deletion causes a relative switch in transcription start sites for *POMP*, predicted to decrease levels of POMP protein and mature proteasomes in terminally differentiated keratinocytes. In order to understand the pathophysiology behind KLICK we created an *in vitro* model of the disease using siRNA silencing of *POMP* in epidermal air-liquid cultures. Immunohistochemical analysis of tissue constructs transfected with siRNA against *POMP* revealed aberrant staining of POMP, proteasome subunits and the skin differentiation marker filaggrin when compared to control tissue constructs. The staining patterns of *POMP* siRNA tissue constructs were strikingly similar to those observed in skin biopsies from KLICK patients. Furthermore, knock-down of *POMP* expression in regular cell cultures resulted in decreased amounts of proteasome subunits and an increase in subunit transcripts. Prolonged silencing of *POMP* in cultured cells induced an increase in C/EBP homologous protein expression consistent with an activation of the unfolded protein response and increased endoplasmic reticulum (ER) stress. The combined results indicate that KLICK is caused by reduced levels of POMP, leading to proteasome insufficiency in differentiating keratinocytes. This disturbs terminal epidermal differentiation, presumably by increased ER stress and incomplete filaggrin processing. Our findings underline a critical role for the proteasome in human epidermal differentiation and suggest that tissue constructs with siRNA induced POMP insufficiency can serve as a model for KLICK genodermatosis *in vitro*.

POSTERS

P01.01
The factors associated with the structure of the public attitude toward the genome research by the latent class analysis in Japan

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The purpose of this study was to clarify the factors associated with the structure of the public attitude toward the genome research by the latent class analysis. The participants of the national wide surveys on the attitude toward the genome research were comprised of 4,000 people (age, 20-69) in 2005 and in 2009, selected from the Japanese general population by using the two-step stratified random sampling method. Five clusters ("Group of aggressive promotion", "Group of passive support", "Group not making judgment", "Group making prudent judgment", "Group not interested in genome".)were assumed as an explanation model of six variables related to the knowledge of genome and attitudes toward genomic research promotion about three themes; basic genome research, genome research related to agriculture and medicine at the survey in 2005. These clusters are associated with academic background, socioeconomic status, to donate to their blood for genome research, interest of science, to want to genome lectures. These factors become the basis of genome research outreach programs.

P01.02
Genetic causes of pervasive developmental disorders

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The pervasive developmental disorders (PDDs), or autism spectrum disorders (ASD), range from a severe form, called autistic disorder, to a milder form, Asperger syndrome.
This paper will focus on classic autism and Asperger syndrome, intending to review the current understanding of the etiologies and the multiple pathogenetic pathways that are likely to lead to the autistic phenotype. Like mental retardation, autism is likely to be caused by many different genetic mechanisms and genes rather than a single, or few, major genes or environmental effects. Applying routine genetic testing in clinical (CLIA-certified) diagnostic laboratories it's possible to identify the specific etiology and recurrence risk in 10% to 15% of autism cases and is clinically indicated for any child with autism.
In this study we report 5 cases with different genetic syndromes in which ASD has been described as one of the possible manifestations: Down syndrome, 22q13 deletion, ,,cri du chat" syndrome - 5p15 deletion, Cornelia de Lange syndrome, Sotos syndrome.
Conclusions: The etiologies of ASD are complex and presence of a wide variety cytogenetic abnormalities are providing us with extremely valuable information about the role played by genetics in autism and collaboration with psychiatrists. Such recognition and understanding will help clinicians implement syndrome-specific treatments of patients identified with a genetic cause of autism spectrum disorder. Early identification of this genetic disorder is critical not only to the individual patient but for the entire family.

P01.03
Assessment of Assisted Reproductive Technology and its associated risk with Autism Spectral Disorder in Indian population

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Autism Spectral Disorders are hypothesized to be on a rise across the globe and developing countries like India are no exceptions. According to unconfirmed estimates there are about 1.7 million affected individuals in India. Increased globalisation and changing socio- economical development seen in last two decades have led to changing lifestyles and increased exposure to various risk factors of Autism Spectral Disorder.
In India, metropolitan cities like Chennai, Hyderabad etc. are showing an increased incidence of stress related disorders like Thyroid malfunction, Polycystic Ovarian Disease, infertility etc., and hence assisted reproduction is on the rise among the young urban population. Though similar indications are available in other countries, the suspected association between increased rate of Assisted Reproduction and Autism Spectral Disorder in India needs to be established.
To explore this link, a questionnaire based case-control cohort study was conducted in Indian Autistic population and preliminary results indicate that ART cases represented 8-10% of the autistic subjects analysed. This is the first report from Indian population indicating the link between Assisted Reproduction and Autism Spectral Disorder.

P01.04
Mutation analysis of familial NF1 pedigrees with multiple affected individuals

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Neurofibromatosis is the one of the most common autosomal dominant inherited disorders that occurs 1 in 3500 live births. The penetrance of NF1 is virtually 100 %. NF1 is characterized by multiple cafe-au-lait spots, cutaneous or subcutaneous neurofibromas, plexiform neuromas, axillary or inguinal freckling, optic gliomas, and iris Lisch nodules. Here we present two families with multiple affected individuals. One of the case was diagnosed as bicytopenia (leukopenia and thrombocytopenia) and splenomegaly. Cases had multiple café-au-lait spots and immobile tumors. Histopathological analysis of tumors diagnosed as neurofibroma. Also ophthalmological examination showed Lisch nodules. Cases are clinically diagnosed as NF1. We performed DNA analysis for family and c2041 C>G p.R681X mutation which is located at exon 13. Second family has six affected individuals, which are diagnosed as NF1 with clinical findings. Also at family history there was two suspicious deaths because of brain tumor without certain diagnosis. DNA analysis of six individuals revealed c.4084 C>T p.R1362X mutation which is located at exon 23.2. These two mutations are defined before at literature. Molecular diagnosis of NF1 is challenging and expensive, but DNA analysis are required for these kind of families which have multiple affected individuals for genetic counseling and further prenatal diagnosis.

P01.05
The participation of minors in biobank research: reflections and recommendations

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The participation of minors in biobank research poses specific ethical questions that are not analogous to those raised by the participation of adults. Specifically, issues regarding parental consent, assent, the question of minimal risk and benefit and the return of individual research results to parents and minors need in depth reflection. For example, should parents be given the opportunity to consent to each possible genetic research on the samples of their children, or should they only be allowed to give specific consent? At which age is a child able to understand genetic research on his or her sample and/or assent to such research? What, if any, risks do children face when they participate in biobank research? Should such research specifically benefit children? Do parents have the right to access all genetic data that are generated by the research? Do researchers have a duty to return individual results that may be of potential health benefit to the child? We have finished a three year project investigating these issues. This project involved reviews, empirical research to query the opinion of lay people and professionals, theoretical reflections and recommendations. In this paper we shall present our findings and especially focus on the recommendations that were the conclusion of the investigation.

P01.06
BRIF: Bio-resource Research Impact Factor. Towards a working tool to promote and recognise sharing of biological samples and associated data.

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A bio-resource can be a database, a biobank, a set of bioinformatics software tools etc. As part of the work on incentivisation to construct, to share and to use bio-resources, the concept of a Bio-resource Research Impact Factor (BRIF) has been introduced. The concept relies on a quantitative parameter for bio-resource use, similar to the Impact Factor for publications. Such a BRIF would make it possible to document: 1. the quantitative use of a bio-resource, 2. the quality and the importance of research results involving it, and 3. the scientific and management efforts of those who set up and made available a valid bio-resource. A working group has been set up towards the creation of this BRIF, starting with an online forum http://www.gen2phen.org/groups/brif-bio-resource-impact-factor in EU projects. The work addresses several steps: 1. Creating a bio-resource unique identifier, or digital ID; 2. Standardising bio-resource acknowledgement in papers; 3. Cataloguing bio-resource data access and sharing policies; 4. Identifying the other factors to take into account when calculating the Impact Factor; 5. Prototype testing, involving volunteer bio-resources and the help of journal editors. Such a system could be used to rationally evaluate bio-resource activities over time. Such a system could be used to rationally evaluate bio-resource activities over time. Specificities in data sharing versus biological samples have to be considered. Also, if taken into account in assessing researchers/contributors professional activity, the use of BRIF would probably promote both quality and sharing of Bio-resources and contribute to harmonising sharing policies.

P01.07
Cafe for Routine Genetic Data Exchange

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Diagnostics laboratories assess DNA samples from many patients with various inherited disorders, producing a wealth of data on the genetic basis of disease. Unfortunately, those data are not usually shared with others. To address this deficiency, a novel system has been constructed that facilitates the discovery and controlled transfer of diagnostic laboratory data to the wider community, via an internet-based 'Café for Routine Genetic data Exchange' (Café RouGE http://www.caferouge.org/).

Actually, diagnostic laboratories are not reluctant to release their data, but merely face practical obstacles: First, their personnel do not have time nor funding to manually submit data to internet depositories such as Locus Specific databases (LSDBs). Second, they would receive no recognition or reward for releasing their data, giving them no incentive. Café RouGE takes account of these real-world obstacles and the needs of diverse LSDBs and aims to minimize the effort required to publish variant data by:

a) Endowing data analysis tools used by diagnostic laboratories with a 'data submission' function that automatically pushes processed data onto the internet

b) Producing a single internet depot (café) to receive and display the existence of these data, with options for controlled download by diverse third parties.

A modified version of the Gensearch sequence analysis package (PhenoSystems SA) now offers this submission functionality, and other tool providers are encouraged to do likewise. Several hundred mutations per month are now released by users of the Gensearch tool alone.

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P01.08
BRCA1 mutation carriers self-reported levels of anxiety, depression, quality of life> 1 year after genetic testing.

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Background: Genetic testing for hereditary cancer is expected to involve an increasing number of individuals in the years to come due to the rapid development in molecular genetics. The main aim of this cross-sectional study was to investigate the long-term psychological effect of being a BRCA1 mutation carrier and compare this to a matched normal population. Secondly, we wanted to explore if losing a close relative in childhood due to cancer or being affected by cancer were associated with symptoms of anxiety and depression or the level of health related quality of life.

Method: Of the 100 eligible participants, 78 consented to participate and were included in the study. Questionnaires were sent to them at least one year after a positive BRCA1 test result had been revealed to them.

Result: Compared to the Norwegian general population, participants reported higher level of anxiety and low level of depression. Mutation carriers who experienced losing a close relative in childhood due to cancer reported higher levels of anxiety and depression and lower psychological health (WHOQOL-BREF) compared to those without this experience. There was no significant difference between mutation carriers affected by cancer and those not affected.

Conclusion: The results from the present study show that living with knowledge of being a mutation carrier may affect anxiety and physical health. Those who had lost a close relative due too cancer during childhood seemed to be a more vulnerable group, and may need extra intervention from genetic counsellors.

P01.09
Does time frame matter? Communicating age-related or lifetime risks in breast cancer risk communication

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Introduction: Many women overestimate their breast cancer risk. Evidence suggests that people fail to adjust their risk perception to account for longer time frames. It has been argued that using narrower time frames (e.g. 10 years) are more appropriate than life time frames. The aim of this study was to compare the effects of presenting risk information on breast cancer in age-related 10 years frames in addition to life time risks on the counselees' understanding and perception of risks, psychological well-being, and intentions regarding breast cancer surveillance.

Methods: In a randomized controlled multicenter intervention trial, unaffected women with a breast cancer family history were recruited. Women received one of two breast cancer risk presentation formats: lifetime risk in frequencies (i.e. X out of 100) (n=63) or life time risks and age-related 10 years risk in frequencies (n=69). Baseline, 2-week and 6-month follow-up measurements were assessed using questionnaires.

Results The added age-related risk led to a more accurate understanding of 10 years breast cancer risk but not of life time risks, and gave women more clarity. The added age-related risk also lowered women's perceived relative risk of getting breast cancer. However, participants evaluated their own risk as less imaginable when expressed as age-related risk compared to life time risk. The addition of age-related risk had no effect on well-being or preventive intentions.

Conclusion: Our results suggest that the addition of age-related risk to life time risk of breast cancer may have an additional value in breast cancer risk counseling.

P01.10***

Comparison of three different formats of breast cancer risk communication: percentages, frequencies and graphical displays

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Introduction: Inaccurate risk perception is a common finding in genetic counseling of women with a breast cancer family history. It is however unclear how risks should best be communicated. It has been suggested that some risk communication formats (e.g. frequencies, graphs) are better than others (e.g. percentages). We compared the effects of three risk presentation formats on the counselees' understanding and perception of risks, psychological well-being, and intentions regarding breast cancer surveillance.

Methods: In a randomized controlled intervention trial, unaffected women with a breast cancer family history referred to three clinical genetic centers in the Netherlands were recruited. Women received one of three breast cancer risk communication formats: lifetime risk in percentages (i.e. breast cancer risk is X%) (n=38), in frequencies (i.e. X out of 100 women will get breast cancer) (n=63) or in frequencies plus graphical display (100x100 human figure icons) (n=91). Baseline, 2-week and 6-month follow-up measurements were assessed using questionnaires.

Results: No differences were found between women who received the percentages and those who received frequencies in terms of cognitive outcomes, psychological well-being and preventive intentions. Neither was there an effect of an added graphical display. In all three groups women were highly satisfied with the risk information provided.

Conclusion: No evidence was found for different effects of percentages or frequencies with or without a graphical display when communicating breast cancer risks in genetic counseling. Methodological and psychological explanations will be discussed.

P01.11

Gender differences in forming intent-to-obtain cancer susceptibility genetic testing - a group-based structural equation modeling approach.

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Objective: Cancer-susceptibility genetic testing (GT) can advance cancer prevention only if it leads to improvements in current screening behaviors. The gender distribution of breast and prostate cancer genetic risk profiles argues for elucidating gender differences in forming intent-to-obtain GT. We use a theory of reasoned action derived model to test for such differences.

Methods: Cross-sectional analysis of 1,824 US adults. Structural equation modeling tested for gender differences in total/direct/indirect effects of *attitudes* towards GT, *knowledge* about genetic diseases and testing, prior *experience* with GT, and *religious involvement* on intent-to-obtain GT.

Results: 765 men and 1,059 women were surveyed. Despite similar willingness-to-test for curable disorders (77%), men showed higher willingness-to-test for incurable disorders (54% vs. 46% in women). Men were significantly more likely to have had prior experience with GT (7.5% vs. 5.1%). Prior experience with GT had a direct positive effect on intent-to-test among men ($b=0.68$; $p<0.01$), but no effect among women ($p>0.05$). For both genders, attitudes towards GT had a significant direct positive effect on intent-to-test ($b=0.536$ for men; $b=0.391$ for women; $p<0.001$) and individuals with high religious involvement held more negative attitudes towards GT and were less willing to obtain GT ($b=-0.020$ for men; $b=-0.017$ for women; $p<0.05$). The model explained 24% of the variance in intent-to-test among men, but only 12.1% among women.

Conclusion: Significant differences exist between men and women in the pathways to formation of intent-to-obtain GT and underscore the need to refine genetic counseling and outreach to account for gender influences on GT behaviors.

P01.12

Cancer Genetic Counselling in Cyprus: Review of the first six years

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Cancer Genetic Counselling in Cyprus was established in 2004. Patients are referred based on type of cancer, family history and age of diagnosis. Referred patients are offered counselling appointments to be appropriately informed of the risks, benefits, and limitations prior to decision making for testing. Results are communicated in person and also in a written report while further appointments are scheduled according to the needs of the patients and their families.

During the six years of CCGC's running, more than 450 families with cancer history were seen. The patients were mainly referred by their oncologists. Among these 400 were breast/ovarian cancer families and 49 were colorectal cancer families.

Genetic counselling for cancer predisposition genes was in general well appreciated. Uptake of genetic testing by patients and their family members were diverse and the response to results varied. The aim of this study is to analyze demographic information, the variability within this patient group in terms of diagnosis, and also the reported reasons for uptake or denial of testing as well as other data.

P01.13

The StoryBank: A web-based resource of digital stories to meet the information and support needs of cancer genetics patients

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The Cancer Genetics Service for Wales (CGSW) offers a range of services such as risk assessment, genetic counselling and genetic testing for people with a family history of hereditary cancer. As a result of the life-long nature of genetic conditions, a fundamental challenge is to meet the ongoing information and support needs of these patients. The internet is increasingly being used as a source of health information and is one mechanism through which this challenge can be met. Patients often want to hear from others that have been through a similar experience and digital stories are particularly useful for transmitting this information.

The CGSW Research Team have created an online collection of digital stories from 23 patients living with, or at risk of familial cancer, about their experience of cancer, genetics and their journey through CGSW. Staff from CGSW have also contributed to the *StoryBank* with stories answering questions about the service such as 'What is genetic counselling?' The stories can be freely accessed on the *StoryBank* website www.cancergeneticsstorybank.co.uk

The *StoryBank* is a web-based resource designed to meet the information and support needs of cancer genetics patients, new referrals, and anyone concerned about a family history of cancer. It provides reliable and engaging information and allows patients the opportunity to hear the experiences of other patients. It is hoped that hearing real stories about people's experiences can help those with, or at risk of, cancer and will raise awareness of cancer genetics amongst the public and health professionals.

P01.14

A french regional pilot experiment for multidisciplinary management of patients at risk of inherited cancers

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Recently, the French national cancer institute (INCA) launched an extramural program, the main goal of which was to integrate, facilitate and improve global risk management for patients at very high risk of cancer, based on their familial history and/or genetic testing result.

Six pilot experiments were supported in six different geographic area of France and received the following mandates : (1) to offer an individualized cancer screening and preventive recommendations

program to at risk patients, using the latest knowledge and individual patient medical history, (2) to coordinate the introduction of the new screening and preventive option procedures in each unit, (3) to insure access to multidisciplinary services, since patients shoud be offered adequate screening strategies and all necessary information and psychological support when prophylactic surgery is discussed and finally, (4) to offer necessary expertise when difficult cases have to be discussed.
Our pilot experiment takes place in the eastern part of France, in Alsace province. We began with the recruitment of a core team dedicated to these new missions comprising : a genetic counselor, an assistant-coordinator, two research assistants and two psychologists. We will present the first steps and will introduce the new tools we have developed to inform patients and specialists of the more recent national recommendation to be applied for follow-up of at risk patients. We will describe how we will assess the compliance to these recommendations of patients and the medical community. Finally, we will discuss issues encountered in the development and maintenance of this program.

P01.15
Do nurses have the requisite competences related to genetics? A systematic review of the evidence.

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The expansion of genetic testing in monogenic and complex disorders has increased the need for all healthcare practitioners to develop genetic competences. Nurses are the largest single group of healthcare professionals and core competences for nurses have been described by the European Society of Human Genetics. The aim of this systematic review was to determine the extent to which nurses are achieving the core competences in genetics. We conducted a systematic search of the published scientific literature held in four relevant databases, using a set of key words including nurs*, competen* and genet*. A total of 269 papers were retrieved, of these 11 (reporting 9 studies) fitted the inclusion criteria and were included in the review. The main themes extracted from the data were knowledge, skills, nurses' perception of the relevance of genetics to their roles, confidence and ethical issues. The evidence indicates that while nurses across a range of countries and practice areas perceive genetics to be important to their roles, generally their knowledge of genetics is low, as is their ability to perform basic clinical skills, such as taking a family history or referring to genetic services. This is reflected in nurses' low confidence in their ability to deal with genetics issues concerning their patients. In addition, some nurses have ethical concerns that genetic testing could cause discrimination against patients. It is clear that, despite calls for enhanced genetics education for nurses for over a decade, nurses are not equipped to deal with the genomics era.

P01.16
Consent for the feedback of individual research results in international consortia: Examples from the International Cancer Genome Consortium

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Returning individual research results to participants is an increasingly debated subject. It is suggested that, in order to be returned, findings should be scientifically valid and confirmed, have significant health implications and there should be therapeutic or preventative options available. However, the process by which these findings will be communicated will necessarily be different depending on the context of the research. Individual research projects are able to rely on available institutional guidance. Consortia whose members are all located in one country may need to deal with different institutional procedures, but will also have national law and regulations to guide their actions. International consortia face the additional problem of having projects that represent different legal, regulatory and cultural requirements for different aspects of the research process, including returning individual research results. Therefore, how should an international consortium deal with this pressing issue? The International Cancer Genome Consortium has created a policy where the consortium itself will not return results, but recognises that local member projects may choose or be required to return results. Model consent language has been created by the ICGC to assist projects as well as other researchers. Sample language from existing ICGC projects shows how the model consent has been adapted to suit local needs as well as those of the ICGC. As more international consortia are created, others may learn from the ICGC experience.

P01.17
Teaching counseling to students

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The intent of the present study is to continue researching whether ethical issues need to be learned for genetic counseling or whether they are already one of the human mind's perspectives for making moral decisions. Embedded in human behavioural patterns, ethical or moral principles look as if they were *inborn*, but it appears that that usually people only get alerted or react when their socially *acquired* ethical or moral rules and values are somehow out of balance compared to those of the surrounding world. If counseling is nothing more than exercising one's own moral concepts, are medical students gaining new ethical principles during their genetic training or are they just consolidating their previous opinions?
The target group in this study consisted of first year medical students who originate from other countries beside Romania. Their social, educational and moral backgrounds differ within the investigated group. To eliminate biases identified in a similar previous study, already during the first practical an article from the specialized literature was read by students and then they wrote down their opinions. The same questions were also answered at the end of their training in medical genetics, the changes of their views being discussed, if any. These new data are compared to those analyzed and presented last year, when only Romanian first year medical students read the same article and answered the same questionnaire.
Increased understanding of the students' views on moral issues can help organizing the teaching process of genetic counseling.

P01.18
Clinical experiences of genetic counselors ordering CYP 2D6 genetic testing for tamoxifen therapy in breast cancer

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Personalized medicine is a field growing in both interest and practice. In terms of breast cancer therapy, genotyping of *CYP2D6* exists to determine a patient's tamoxifen metabolism. Despite the high incidence of breast cancer in the United States, the frequency with which tamoxifen is prescribed for treatment, and the shift towards personalized medicine, no data exists of the frequency of ordering CYP 2D6 genotyping in a genetic counseling setting, or the barriers that are faced when ordering the test. The purpose of this study was to identify both the frequency of genetic counselors ordering CYP 2D6 genotyping and the barriers that they faced - both experienced and perceived. An online survey was distributed over the NSGC Cancer SIG Listserv. Thirteen percent (n = 6) of respondents had ordered CYP 2D6 genotyping for a patient. Sixty-five percent (n = 30) of respondents provided information regarding barriers they faced when ordering the test, including uncertainty of clinical utility (77%), not my role/role of the oncologist (60%), unfamiliarity with the test (40%), recommendations of professional societies (40%), and lack of impact on patient's medical management (20%). Literature suggests that personalized medicine is the future of oncology; however this study found that genetic counselors have little experience with CYP 2D6 testing.

P01.19**
Cystic Fibrosis carrier screening: customer satisfaction study

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We have been performing preconceptional identification of Cystic Fibrosis (CF) heterozygotes since 2001. 13168 subjects were analysed and 400 carriers found.
We refined the distribution of the different CFTR mutations in our population, defining a more sensitive and less expensive mutation

panel. To assess the impact of this test on the carriers, we performed 100 phone interviews. Even if the couples were followed by gynecologists and family doctors and received an informative leaflet and a written report, the majority of the carriers didn't know what CF was and what a carrier status meant. The majority of the women were prescribed the test during the pregnancy and not before, and there isn't an agreed protocol for testing (both members at the same time or one member first). Only 39% of the carriers decided to have a second level test for their partner, and the 12% of them got a positive response too. This five couples were not influenced in their reproductive choices by the positive test, and asked for a CF pre-natal test for all their pregnancies, with no double heterozygote babies to date. The 61% of carriers that did not screen the partner, did not report the birth of CF babies. Even if the interviewed carriers were happy with the test and suggest to their friends and family to be tested, the results of the questionnaire forced us to change the way we deliver the report and to improve the dissemination of informations among the physicians and the public.

P01.20

Genetic counseling in phenylketonuria (PKU) and cystic fibrosis (CF) patient's parents

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Mandatory newborn screening for PKU is spent in Russia since 90th years of XX century, and for CF since 2006. To estimate patient's parents opinion concerning different aspects of genetic services we have spent questioning of 187 PKU and 93 CF patient's parents. PKU patient's parents more often have learnt about the hereditary nature of their child disease from geneticist than CF patient's parents (75% and 51% respectively, $p<0.05$). CF patient's parents more often than PKU patient's parents have noted that they have not understood the information on repeated genetic risk (6.4% vs 1.2%, $p<0.05$) and incorrectly specified its value (32.0% vs 15.0%, $p<0.05$). However they more often regarded genetic risk as high whereas PKU patient's parents more often - as average ($p<0.05$). Prenatal diagnostics was more acceptable for CF patient's parents (64.9% vs 43.3%,$p<0.05$). Only 1.1 % of respondents have answered that they didn't want to terminate the CF foetus pregnancy, and 23.5% PKU patient's parents have selected the same answer ($p<0.05$).

P01.21

Cystic Fibrosis and Sickle Cell Diseases: Young adults' understanding of newborn screening

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Recently implemented Newborn Screening (NBS) for Cystic Fibrosis (CF) and Sickle Cell Diseases (SCD) across the UK raises questions about the public's level of awareness of NBS. Pregnancy is believed to be a difficult time to communicate about NBS, as information is poorly absorbed and negative consequences may arise for parents during pregnancy and after birth. Although awareness of NBS is suggested to reduce the distress associated with screening, research fails to stipulate what information is required or when it should be provided. Furthermore, the extent of lay disease knowledge prior to screening is not established. This study explored i) young adults' existing knowledge of CF, SCD and NBS, ii) how awareness of NBS can be improved for future generations and iii) the feasibility of providing NBS information to a young adult population. Thirty four young adults took part in one of seven focus groups. Thematic analysis was conducted to generate codes and themes at a manifest level. Coupled with numerous misperceptions, students displayed limited knowledge of CF and SCD. Predominantly informed by Biological education, awareness of the severity of SCD was typically underestimated. Participants dismissed wanting further NBS information during young adulthood despite showing preoccupation with personal carrier risk. Lack of knowledge and perceived irrelevance of screening information causes concern about how to prepare the future generation of NBS programme users. The inclusion of carrier statistics, autosomal recessive inheritance and information about the severity of SCD are proposed to improve young adults' interest and understanding of NBS.

P01.22

Looking Forward: Involving young people with Cystic Fibrosis (CF) in developing an educational arts package exploring CF and gene therapy

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Cystic Fibrosis (CF) is one of the most common genetic conditions in the UK, affecting approximately one in 2500 people. Patients with CF cannot meet up in person due to the risk of infection; therefore their opportunities to discuss the latest scientific research related to their condition can be limited. Gene therapy is often heralded as a potential future treatment for CF and first phase clinical trials are underway in the UK. Despite the frequent news headlines reporting continuing progress, the consistent and effective future use of gene therapy remains only a possibility.

The *Looking Forward* project brings young CF patients, clinicians, artists, scientists and educationalists together to develop an online educational arts package exploring CF and gene therapy. Young CF patients are brought together via web-based technologies such as social networking websites and videoconferencing, and will also work in one-to-one sessions with members of the project team. Creative and novel approaches will be used to explore the science behind gene therapy and the personal, social and ethical issues associated with this field of research. The resulting resource will be disseminated to a wide audience, including CF patients and their families, school pupils, teachers and health professionals. Importantly this project will explore methods of participatory engagement with individuals who cannot meet face-to-face (the CF patients).

This project is a unique collaboration that puts young people with CF at the centre of planning an educational arts package to ensure that the resource created is valuable and meaningful.

P01.23

The views of CF patient's parents on prenatal diagnostics

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To estimated views on prenatal diagnostics (PD) we have spent questioning of and 93 CF patient's parents. Majority of respondents (83%) knew about the PD of CF, and 52 % have received this information from geneticist; 42 % from them answered, that the possibility of PD has changed their reproductive plans. The majority of parents (81 %) believed, that PD of CF is necessary procedure, 78 % believed, that it could solve family problems and for 65 % of them PD is acceptable. More than 50 % of respondents would terminate pregnancy with CF foetus. To reveal factors which can influence the relation to PD Spearmen rank correlations were calculated. Correlation of reproductive plans changing with burden of treatment of CF child, and storing the value of repeated genetic risk was shown. Acceptance of PD was correlated with the age and educational level of woman, whether or not she was referred for genetic counselling, and whether or not she has received an explanation about repeated risk. Correlation of reproductive plans changing and acceptance of PD was shown too.

P01.24**

Dravet syndrome caused by SCN1A mutations, and other genetic causes of epilepsy, with onset after vaccination in infancy

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Background: Vaccinations are one of the most cost-effective health interventions of the 20th century. Alleged adverse effects, like (a)febrile

seizures, may have great impact on public acceptance of vaccination programs. In this study we investigated the etiology of epilepsy following vaccination, in a national cohort.
Methods: Medical data of 1301 children, reported with convulsion(s) after vaccination in the first two years of life between 1997-2006 to the National Institute for Public Health and Environment in the Netherlands, were re-evaluated. Parents of children with onset of epilepsy within 24 hours after a DTP-IPV-+Hib vaccination, or 5-12 days after MMR-vaccination were re-contacted for follow-up.
Results: Twenty-seven children (2,1%) had epilepsy with onset after vaccination. Follow-up was available for 24. Ten children had a genetic cause: Dravet syndrome due to a SCN1A-mutation (8), epilepsy in females with mental retardation (EFMR) due to a PCDH19-mutation (1) and a submicroscopic 1q deletion (1). A genetic cause was presumed in four others: neuronal migration disorder (2) and autosomal dominantly inherited form of epilepsy (2). Of the remaining 10 children, three had a first or second degree relative with seizures. In one child the cause of the epilepsy was probably viral.
Conclusions: In the majority of cases with epilepsy onset following vaccination in the first two years of life, a genetic cause is detected or suspected (14/24). Early diagnosis of Dravet syndrome and other genetic causes is not only important for treatment and genetic counselling, but also for safety surveillance of vaccination programs.

P01.25
Views of relatives of children with Duchenne muscular dystrophy on their experience of diagnosis and population screening

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Newborn screening (NBS) for Duchenne muscular dystrophy (DMD) is controversial as there is no treatment that can be implemented at birth, and the pros and cons are subject to debate.
We conducted a qualitative study exploring relatives' views on population screening for DMD in Australia. Participants were recruited through the Muscular Dystrophy Association and RCH Neuromuscular Clinic. A total of 13 families were interviewed: 15 parents, 1 grandmother and 2 adult sisters. Parents reported a 1-2 year gap between their first noticing signs of developmental delay and of receiving the DMD diagnosis; all found this delay in diagnosis to be very difficult. Most participants perceived population screening for DMD to be acceptable, preferring infant screening (6 months to 2 years) but generally opposing NBS. Two parents only felt resources would be better used for improving treatment. The main reason against NBS was potential for a negative impact on parental-child relationship. Participants supporting infant screening felt that a diagnosis in infancy would allow: implementation of early interventions; guidance with financial, practical and reproductive decision-making; and greater understanding of the child's struggle with developmental milestones. Some parents supported a more 'targeted' approach to population screening of infants at ≥ 1 year of age, the time at which most started to be concerned with their child's development. Based on these and our preliminary audit data (showing a median age of 5 years at testing for 70 boys [<18yr] in the 2005-2009 period), we are currently developing various models of offering population screening.

P01.26***
Genetic counsellor education and professional standing in 17 European countries

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Quality genetic healthcare services should be available throughout Europe. However, due to enhanced diagnostic and genetic testing options, the pressure on genetic counselling services has increased. Appropriately trained genetic counsellors and genetic nurses can offer clinical care for patients seeking information or testing for a wide range of genetic conditions and the European Society of Human Genetics is making efforts to set up a system of accreditation for genetic counsellors, to ensure safe practice. We undertook a descriptive, cross-sectional survey to obtain baseline data on the role, education and practice of genetic counsellors and nurses in European countries. To collect the data, we approached a number of key informants (leaders in national genetics organisations or experienced practitioners) to complete an online
survey, reporting on the situation in their own country. Twenty-eight practitioners responded, providing data from 17 European countries. The findings indicate huge variation in genetic counsellor numbers, roles and education across Europe. In United Kingdom and the Netherlands there are more than 4 counsellors per million population, in five countries there is less than one counsellor per million, while in Turkey, Czech Republic and Germany there are no counsellors. There are specific educational programmes for genetic counsellors in seven countries but only France has a specific governing legal framework for genetic counsellors. We will present these and other data to demonstrate the disparity in approaches to the education and use of genetic counsellors across Europe. This study underpins the need for a coherent European approach to accreditation of counsellors.

P01.27
Deficiency of genetics standards for secondary education in the United States

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Recent changes in federal oversight of science education in the U.S. have imposed testing and accountability requirements at the national level through the No Child Left Behind Act (NCLB). Implementation of NCLB relies heavily on learning outcomes, or "standards." Those standards, in turn, drive curriculum and instruction. However, unlike many European countries where the science curricula are also national, in the U.S. curriculum is established at the state and local levels. Given the centrality of standards to teaching and learning, we investigated the quality of life science/biology standards with respect to genetics for all 50 states and the District of Columbia using core concepts developed by The American Society of Human Genetics as normative benchmarks. Our results indicate that the states' genetics standards, in general, are poor, with more than 85 percent of states receiving overall scores of 'inadequate'. In particular, the standards in virtually every state have failed to keep pace with changes in the discipline as it has become genomic in scope, omitting concepts related to genetic complexity, the importance of environment to phenotypic variation, differential gene expression, and the differences between inherited and somatic genetic disease. If the genetics standards (and, by inference, the curriculum) are not improved, the deficiencies we identified may adversely affect genetics instruction and learning, the preparation of future genetics researchers, and the genetic literacy of the U.S. citizenry as medicine becomes more informed by genomics.

P01.28
Enhancing knowledge about modern genetic medicine amongst secondary school students and their teachers in the UK

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The *Nowgen Schools Genomics Programme* aims to change the way that genetics is taught in UK secondary schools. This three-year programme started in July 2009, funded by the Wellcome Trust, and has made significant progress to date. The multidisciplinary team use a range of approaches to help equip young people to assess the real potential of genomics, and to make informed decisions about future healthcare. This paper highlights three successful elements of the programme.
A series of broadcast educational films have been produced to enhance school science lessons and promote awareness of genetic medicine amongst teachers. They feature researchers explaining their

investigations on the role of genes in complex diseases. The films reflect many areas of uncertainty and the great challenges involved. Personal accounts are also given by some patients, who describe the real impact of the conditions. See films at: www.teachers.tv/series/genetics-and-medicine.
Two events were run with 25 researchers working with small groups of school students (aged 16 and 17) to give them a unique insight into the world of research. The students met researchers in their laboratories and found out about latest technologies and methods used in genetics and genomics research. Students also spent part of the day considering the wider implications of genetics research and bioethics.
One of the most important achievements of this work programme has been its influence on genetics taught in schools in England and Scotland. The project team has worked closely with curriculum developers and made a significance impact on the new school curricula.

P01.29
Nowgen's professional training courses in bioinformatics

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Nowgen - A Centre for Genetics in Healthcare develops and delivers an exciting portfolio of training courses for academics, health and industry professionals. Nowgen's portfolio of training courses covers a wide range of topics including: ethical and social aspects of genetic research, pharmacogenetics, cancer, molecular biology next generation sequencing and bioinformatics.
Modernising Scientific Careers (MSC), led by the Chief Scientific Officer at the Department of Health, is a key programme designed to re-model career pathways for healthcare scientists. Nowgen with colleagues at NW e-health in The University of Manchester, the National Genetics Reference Laboratory (NGRL), Manchester, alongside the professional bodies' bioinformatics working group, are developing the MSC bioinformatics curriculum, in particular for genetics trainees and the wider life sciences MSC programme.
In addition, together with the NGRL and NW e-health, we are delivering a programme of bioinformatics training for healthcare practitioners and scientists in genetics, including: short bioinformatics courses for cytogeneticists, molecular geneticists and clinical geneticists, and the development of a distance learning module to complement the current short bioinformatics course for scientists.
We are also delivering next generation sequencing bioinformatics training for researchers, and clinical and industry based scientists: *Next generation sequencing bioinformatics* will be held on 14 June, 2011. This course is being delivered in collaboration with Genomatix, Life Technologies, Roche and Illumina.
For further information on Nowgen's bioinformatics courses contact: Tom.hancocks@cmft.nhs.uk

P01.30
Geographical origin and genetic isolation in southern Morocco

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The study of the endogamy provides information about the repartition, the structure and the heterogeneity of the genetic heritage of populations.
The main aim of this study is the evaluation of the level of geographical endogamy in the population of Souss-Massa-Draa in south of Morocco. This work consists in a prospective survey of 277 families sampled at random from October 2005 until April 2007. From survey's data, the choice of the spouse is studied according to the origin and the place of birth of the husband and the wife in the generation of the couples and in their parents' generation.
The results clearly showed tendency of couples to the geographical endogamy. In deed, in 83% of studied couples, the spouses chose each other from the same origin whereas in parents' generation, the choice of common origin represented 95%. Moreover, the intergenerational comparison of measured endogamy rates gave the same degree of genetic isolation for both studied generations ($p>0.05$).
In comparison to other results obtained in other regions of morocco, the region of Souss-Massa-Draa is considered to be closed.

P01.31
Break oder continuity? Human genetics in Germany after 1945.

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In 1945 Human Genetics was not established at German universities and efforts were made to change this. After 1933 founded institutes for 'racial hygiene' or 'human heredity' did no longer exist. Therefore well-known people in the field of Human Genetics could carry on their research: 1946 Fritz Lenz in Goettingen, 1948 Wolfgang Lehmann in Kiel und 1951 Otmar von Verschuer in Muenster, in neurologic and psychiatric research supported by Gerhard Koch. The number of institutes increased up to the year 1980 to 24.
Those named Human Genetics can be called the **Pre-War-Generation**. They have in common their relationship to the *Kaiser-Wilhelm-Institute for Anthropology, Human Heredity and Eugenics*, as director (Verschuer), members (Lenz, Becker, Lehmann) or guest (Koch).
• Because of lack of scientists after 1945 the Pre-war-generation got a second chance. They continued with their research on 'old ideas'.
In 1960 the *Wissenschaftsrat* (German Science Council) stated that new professorial chairs at universities should only be for fields that were still in evolution. In medicine a chair for Genetics at every Medical faculty was regarded necessary, replenished by a chair for anthropology.
Right afterwards Helmut Baitsch was appointed professor in Freiburg (1961), Widukind Lenz in Hamburg (1961) und Friedrich Vogel in Heidelberg (1962). Those belong to the **Post-War-Generation** of German Human Genetists. They set the stimulus for the development and professionalization of Human Genetics in Germany. The institutionalisation was supported by public policy.
• The break came around 1960. The Post-war-Generation established Human Genetics at German universities.

P01.32
New Bioethics Law in France regarding human genetics: evolution, revolution or stagnation?

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France is in the process of revising its bioethics law 5 years after its last revision (2004). This law addresses genetic testing among other aspects (e.g. embryo stem cell research, transplantation, medical aided procreation). In various French legal instruments genetic information is treated apart from other biologic information given its potential misuses and its familial dimension. The proposal for revising the law is focusing on two major points of genetics: communication of the genetic Information and optimisation of laboratory's quality. Based on professional secrecy medical doctors could not inform, on their own decision, family members about genetic characteristics, even if relevant for them. New procedures to allow medical doctors to disclose such information to family members are proposed; liability incurred in case of non transmission of the information and the role of patients associations to deliver relevant information are underlined. Although provisions on quality aspects in a bioethics law can be discussed, they aim to protect people through a high quality of laboratory tests and analyses performed, with regards to direct to consumer genetic tests on internet. A key role will be devoted to the "Agence de la Biomédecine" for assessing quality of laboratories and delivering information regarding such tests. Many other aspects have been discussed in the preparatory debates to the revision, such as consent issues (for deceased people) or biobanks and are absent from the proposal. However these issues are among the most problematic in research and practice. We will discuss the consequences of this lack of regulation.

P01.33
The Not So „Incidental“ Research Results

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Research itself has always been understood as the search for generalizable knowledge and not concerned with the individual per se. Today, the ability to perform whole-genome sequencing (WGS) at a fraction of what it used to cost is about to change the “no returns” policy of both longitudinal, population studies and some disease-specific cohorts. Moreover, as researchers begin to access such population biobanks for disease-specific studies using (WGS) or, when begin using WGS in their own clinical research, the concept of “incidental” findings (ie. unforeseen and unrelated to the disease under study) may also be challenged, (if not become obsolete), as the whole genome of a person is exposed. The classic duo of returning only those results with scientific validity and clinical utility is no longer a bulwark to refusal to communicate when ethics guidance requires that any results with “significant welfare implications for the participant, whether health-related, psychological or social” be returned. This raises the issue of which results exactly should be returned, when, by whom, and, to whom (if at all).

Unless the delineation of responsibilities is clarified, it will be health professionals and researchers who may be the most “at-risk” from incidental findings. Indeed, the no-returns policy for research results that includes the not so “incidental” result may no longer be acceptable. How to delimit this seemingly, open-ended avenue of potential professional responsibility and possibly, liability? What are the mechanisms available?

P01.34
Assessing the Assessment Tool: is the ELSI component of ACCE adequate?

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Genetic tests for susceptibility to common, complex disorders are proliferating, both in clinical and direct-to-consumer (DTC) settings. However, many of these tests, focusing on genes involved in multifactorial disorders, have limited clinical validity and utility. Due to the nature of the genetic information that these tests provide, coupled with the relative lack of end-user genetic information literacy, it is especially important that the quality of these tests (in both a clinical and DTC setting) be independently evaluated. A handful of tools and initiatives have been proposed to aid in the evaluation task. One such tool is the ACCE model; consisting of 44 questions, designed to assess the quality of a genetic test’s **A**nalytical validity, **C**linical validity, **C**linical utility and also, to address **E**thical, legal and social issues (ELSI) of the test. Of the 44 questions, three have been allocated to examine the ELSI. Although literature exists that discusses the adequacy of the ACC components in ACCE, little has been elaborated for the ELSI questions. As such, we used a literature review and a psychiatric genetic test case study to critically analyse the adequacy of the ELSI component of ACCE. Our analysis identified some potential gaps in the ELSI component of ACCE and we discuss how these may be reduced. The ELSI of genetic tests are particularly challenging and important, given among others, the possibility for the information to be simultaneously individual, familial and communal, and the short- and long-term impact it can have on all these three levels.

P01.35
Ethical dilemmas in predictive testing: challenges for genetic counselors

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Predictive testing protocols are intended to help patients affected with hereditary diseases understand their condition and make informed reproductive choices. However, predictive diagnosis process may expose clinicians, consultands and families to ethical dilemmas that interfere with genetic counseling and the decision making process. We describe ethical dilemmas in a series of four cases involving predictive testing for hereditary ataxias in Cuba and Portugal. Ethical challenges demand a multidisciplinary approach, where genetic counselors, psychologists, social assistants, medical doctors and other health professionals integrate the psychological, socio-familial and medical issues of complex decisions surrounding predictive testing. The clinical scenarios presented and the general discussion could be useful for health professionals involved in predictive programs for hereditary ataxias and other neurodegenerative disorders.

P01.36
Ethics of personal genetic profiling

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Following a two-year inquiry, the Nuffield Council on Bioethics, a UK independent advisory body, has published a report which explores the ethical issues raised by new developments in ‘personalised healthcare’, including commercial personal genetic profiling. The Council found that these services have a number of potential benefits, such as providing reassurance or enabling people to take preventative action. But potential harms were also identified. For example, the test results can be unreliable and difficult to interpret; ‘good’ results may lead to complacency in lifestyle; learning about risk of disease could be upsetting, particularly if no treatments are available; there is potential for misuse of personal genetic information; and people may seek unnecessary further tests or advice from their doctor. However, the number of people using genetic profiling services and whether this is currently leading to any actual harm is not known.

Several ethical values come into play, such as individuals’ ability to pursue their own interests and efforts by the state to reduce harm, and the report explores the extent to which genetic profiling tests really do lead to healthcare becoming more ‘personalised’. On the basis of this, the Council makes a number of recommendations for policy. For example, regulators are urged to request evidence for any claims being made by companies about the clinical value of their tests, and governments are encouraged to provide information on their websites about the risks and benefits of personal genetic profiling, including the relevance for insurance.

P01.37
Are published genomic data free for further uses? Legal and ethical issues in a context of data sharing

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The use of published information and data for generating electronic databases, such as LSDBs (locus specific databases), for meta-analyses and for new association studies is frequent. Current policies towards data sharing encourage access to and circulation of data. Nevertheless ethical issues are raised regarding informed consent of the person from whom the data were collected. Published, hence publicly available data are not necessarily data free to be used. Limitations given through the consent procedure may exist and the fact that data are published does not erase them. The problem is growing with the rapid technological advancement and more control by the citizens about the use of their data. Those issues are new challenges in the frame of the revision of the EU directive 95/47 on the protection of personal data. We analysed various possible solutions and studied the associated responsibilities of researchers, institutions, editors, ethics committees and data mining scientists. This reflexion led to propose to systematically indicate in the publication itself or as supplementary information possible other uses of data/samples included in the informed consent and other limitations and to ask the editors to consider introducing this in instructions to authors. It would provide useful information to potential users in order for them to decide whether to use the published data or not. It would be respectful of privacy rights and autonomy. Nevertheless it remains limited in cases where the consent is not indicating the possible further uses of data. Other solutions can be proposed in such cases.

P01.38
Secondary use of research data: ENGAGE and BioSHaRE as proof of concept

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Biobanking activity is increasingly at the heart of research practice. Access to such resources is of major interest for research since it avoids the limitations of research conducted among smaller number of subjects. Alongside these benefits, however, remain the ethical issues of respect for the autonomy and privacy of research participants, most notably in the context of secondary use of data, i.e. where data is used for purposes falling outside the scope of the consent given by research participants.

The last decade has witnessed the attempt to establish the ethical acceptability of the secondary use of data. It has remained unclear however as when should research accessing biobanks be considered as "primary" vs. "secondary" use, and as to how to apply these criteria in the context of international research consortia such as ENGAGE and BioSHaRE.

The European Network for Genetic and Genomic Epidemiology (ENGAGE) is a research project performing meta-analyses in order to translate the wealth of data emerging from large-scale research in genetic and genomic epidemiology from 23 population cohorts into information relevant to future clinical applications.

The goal of BioSHaRE is to allow researchers to use data pooled from different cohorts and biobank studies, thereby creating sample sizes large enough to investigate questions relating to multifactorial diseases.

In order to determine whether the use of already collected samples and data in international research consortia should be considered as either "primary" or "secondary" use, both the ENGAGE and BioSHaRE consortia will serve as models for our study.

P01.39
Medical ethics and counseling genetic in SMA

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Spinal muscular atrophy (SMA) is one of the most common autosomal recessive diseases, affecting approximately 1 in 10,000 live births, and with a carrier frequency of approximately 1 in 50. The SMA phenotype is widely variable, with the severity of symptoms influenced by several genetic factors. Genetic risk assessment is an essential and integral component of SMA genetic testing and impacts genetic counseling both before and after genetic testing is performed.

To better understand the ethical difficulties associated with a condition like SMA, we report a couple where the man was diagnosed with SMA type IV. He has a family history of SMA. Couple was provided information on SMA, including range of severity and test limitations. Parents was requested prenatal diagnosis because they did not accept residual risk.

More, it is almost certain that other father's family members are also carriers of genes mutation. Should the counselor be expected to break confidentiality to inform the at-risk family members of their genetic risk? Comprehensive SMA genetic testing, comprising PCR-RFLP assay, SMN gene dosage analysis and genetic counseling, offers the most complete evaluation of SMA patients and their families at this time. These cases illustrate that SMA testing, like other genetic testing, can lead to complicated scenarios and highlight the importance of genetic counseling for identified SMA carriers. A genetic counselor could help this couple to understand the risk for own children and to develop strategies to accurately inform their family members of their possible risk of SMA without being alarming.

P01.40
Personal genome testing: Characteristics of the test, the disease and the context to clarify the discourse on ethical, legal and societal issues

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Background: Personal genome tests are currently marketed directly-to-consumer to estimate genetic risks for a range of multifactorial diseases and other traits. These tests have spurred critical discussions of their ethical, legal and societal implications (ELSI), but not all personal genome tests raise the same ELSI. We offer a systematic approach towards questions regarding what ELSI to expect from specific applications, and why.

Methods: Review of the bioethical literature, expert interviews and conceptual analysis.

Results: ELSI may vary with the characteristics of the test, the disease and the context in which testing is offered. For example, tests of limited clinical validity are less likely to be utilized as a basis for discrimination than do those of higher levels of clinical validity. Further, testing for a multitude of diseases simultaneously brings along an unequalled quantity of information, thereby threatening the feasibility of informed consent. Also, testing for psychiatric diseases such as clinical depression, is surrounded by various psychological and ethical sensitivities, which are much less pressing in testing for (most) somatic diseases. A major context characteristic is direct-to-consumer access to personal genome testing, which is associated with ELSI of its own, such as lack of professional medical guidance. We have composed a comprehensive list of characteristics that are of relevance to the ELSI-debate.

Conclusion: In an informed and meaningful ELSI-debate on personal genome testing for multifactorial diseases, consideration of characteristics of the test, disease and the context is indispensable, for these characteristics determine the applicability of ELSI to specific personal genome tests.

P01.41
Patients' experiences and views of DNA testing and familial cascade screening for Familial Hypercholesterolemia (FH): a qualitative study

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Familial hypercholesterolemia (FH), an autosomal dominant disease, manifests as a disorder of cholesterol metabolism. Early detection and treatment with statins can be effective in reducing morbidity and mortality from CVD. Familial cascade DNA screening for FH mutations is a clinical reality throughout Europe. Questions have been raised about the most efficient and cost-effective way of implementing cascading. The argument for direct (clinician-mediated) versus indirect (patient-mediated) cascading is that the former overcomes patients' reticence about disclosure and thus, maximises the number of relatives approached and captured in the cascade. However, research suggests that family communication about genetic testing takes place without a need for this intervention. Moreover, direct cascading is reliant upon the index patient revealing family contact details, and this may raise issues about confidentiality for some. To date no research has been undertaken on the views of patients involved in indirect cascading. This paper looks at patients' experiences of DNA screening and mediating familial cascade screening. The data were gathered in in-depth interviews with 38 patients who had DNA testing in lipid clinics SE Scotland. 15(39%) were referred for cascade appointments in clinical genetics. Patients appreciate DNA testing being undertaken by known and trusted lipid clinic staff. DNA testing was seen as part of the normal lipid clinic routine. Having a genetics consultation to identify relatives for cascading was perceived as facilitating, and potentially increasing, family communication about FH. Patients expressed a preference for indirect cascading, commenting that it may be less threatening for relatives than unsolicited clinical contact.

P01.42
Conflicts regarding genetic counseling for Fragile X syndrome screening: A Survey of clinical geneticists and genetic counselors in Israel

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Although Fragile X screening has been offered in Israel since 1994, issues related to potential neurological and gynecological symptoms in carriers make counseling for Fragile X different from recessive disorders. We evaluated the attitudes of geneticists regarding genetic counseling given to the women undergoing screening. We performed a self-administered questionnaire including 13 study questions was mailed to all geneticists in Israel. The questions were

related to counseling for women pre and post screening regarding themselves and the affected fetuses (including the risk for Premature Ovarian Insufficiency; FXPOI and Fragile X-Associated Tremor Ataxia Syndrome; FXTAS). Out of a total of 80 geneticists 34 responded with no additional responses on e-mail re-call. There was no clear consensus for 11/13 (85%) presented questions. The most striking differences in opinion were observed for issues regarding FXTAS risk in prescreening counseling sessions ($p<0.05$).This study demonstrates that , there is no consensus on critical variables implying risk for fetus and mother and that counseling practices are dissimilar even in this small cohort of experts. We demonstrated a conflict between the detailed amount of information which should be given prior to the test in order to allow informed decisions and the overload of information which may cause confusion.

P01.43
The use of genealogy databases in genetic health care

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Background: The use of electronic genealogical databases could facilitate the construction of accurate and extensive pedigrees in genetic health care. Genealogical databases could be linked to specific disorder databases in order to increase the accuracy of pedigrees and inform the genetic risk assessment.
Aim: Systematic review of the published literature on the use of genealogy databases to construct pedigrees for risk assessments in genetic health care.
Design: A systematic literature search was undertaken using the following 12 combined search terms: “Genealogy database* AND genetic risk AND family history”, “Genealogy database* AND clinical genetics” , “Genealogy Database* AND medical AND family history”, “Cancer registry AND genetic service”, “Cancer registration AND clinical genetics AND genealogy”, “Database* AND family history AND genetic risk assessment”, “Genealogy database* AND cancer registry”, “Database* AND genealogy AND genetics”, “Genealogy OR heraldry AND database”, “Genetic counsel* and clinical genetics AND genealogy” and “Cancer genetic counsel* and genealogy”.
Data sources: EbscoHost, PubMed, Web of Science, Ovid, the Grey literature and reference lists of identified studies.
Results: Of 683 titles identified, one study described experience of using a genealogy database in a cancer registry in support of genetic health care. Two more papers discussed the potential use of genealogy databases. **Conclusions:** Limited published information exists on the use of genealogy databases in genetic health care although numerous such databases exist. This may reflect legal or regulative restrictions based on autonomy or information risk. Studies are needed to address the benefits and drawbacks of using genealogy databases in genetic health care.

P01.44
Genetic testing of children: report of a working party of the British Society for Human Genetics

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Genetic tests can offer immediate clinical benefits and should be utilised in the same way as any other investigation used to determine the best clinical management of a child. But, genetic tests can also generate information about children’s health in the medium to long term future, rather than about current health problems. Decisions about the optimum time to carry out a genetic test can therefore raise difficult issues for health professionals, for parents and for children and young people themselves. In 1994 a report from the UK’s Clinical Genetics Society recommended that predictive genetic testing was appropriate where a medical intervention was available and would usually be offered during childhood, but that such testing should not generally be undertaken for adult-onset disorders unless there were clear cut and unusual arguments in favour in a particular case. The report concluded that it was important to preserve or maximise children’s future choices where no clear benefits would accrue during childhood. The guidance has recently been reviewed by a working group on behalf of the British Society for Human Genetics, and a new report was published in 2010. This was informed by research, and took into account developments including other guidelines published over the last 15 years. The report includes recommendations about genetic testing of children, an explanation of the legal (in the UK) and clinical rationale for these and case studies to assist professionals in their use of the recommendations.

P01.45
Fourteen year’s experience of genetic counseling for hereditary hearing loss patients in Iran

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Diagnosis and estimation of recurrence risk are components of a complete genetic evaluation of a child with hereditary hearing loss (HHL). The disease can be classified into syndromic or nonsyndromic forms. The most common pattern of inheritance in HHL is autosomal recessive (AR) with a 25% risk of transmission to the next generation. The aim of this study was to provide more accurate genetic information using in genetic counseling for Iranian HHL families.
Since 1996, genetic counseling has been provided for over 2650 deaf families. Majority of them had 2 and more affected child. Over sixty percent of the families who referred to us had consanguinities marriages. Out of 2650 families, 80% had AR pattern of HHL, 1% AD and X-linked, and the remaining cases were sporadic cases. Nonsyndromic and syndromic forms of HHL account for 79% (n=2094) and 9% (n=238) of families, respectively. Most of the deaf patients had congenital HL (n=2065). The mutation in *GJB2* accounted for overall 16% of the referred families; however, the prevalence of the mutation in this gene significantly varies among different ethnic groups in Iran. In Northwest of Iran the frequency was about 30% while in the East and South was as low as 4%.
We have been able to provide result for 30 to 50% of the referral families depending to ethnic population.
So far, we have provided genetic diagnosis, carrier detection, and recurrence risk estimation for 1500 families to help them in decision making for future pregnancies.

P01.46
The use of iPad in genetic counseling

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Accurate information about genetic aspects of disorders is needed in genetic counseling. To address this need and enable clear and easily understood explanations, professionals in genetic health services have developed a number of educational aids. These aids can be used to inform the counselee of the inheritance pattern, risk to relatives, genetic testing options, symptoms and surveillance. They can also be used as decision aids regarding genetic testing and prevention. Most often these aids are visual; images, booklets, interactive computer-based programs, checklists and CD/DVD´s,
It has been shown that counselees using educational aids before or during genetic counseling session are more likely to remember their risk status, show significant knowledge improvement and are more satisfied with their decisions. However, the use of educational aids has not been shown to benefit the psychosocial aspects of genetic counseling and counselees prefer personal communication.
Recently, touch screen computers such as iPad, have made their way into the medical world. We have introduced the use of iPad in genetic counseling at The National University Hospital of Iceland. With an

iPad the genetic counselor can show the counselee defined packages of information, including small videos, and use the touch screen to draw further images for explanation. The flow of information can be controlled and it is possible to zoom in on images. The use of iPad combines the benefit of personal communication and an educational aid in genetic counseling to improve the overall experience.

P01.47
The medium and the message: Genetic counselling in the direct-to-consumer genetic testing industry

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As part of a larger study of direct-to-consumer genetic testing (DTCGT) for psychiatric disorders, we investigated the role genetic counselling is coming to play in the DTCGT industry, and its implications for how genetic counselling is understood and provided, addressing a common critique of the DTCGT industry (Hudson et al. 2007; Nuffield Council on Bioethics 2010). We performed qualitative analyses of two web-based resources (a sample of DTCGT websites and genetic counselling blogs and forums), to answer the following research questions: with what types of genetic testing products is genetic counselling offered; what forms of genetic counselling are offered; how is genetic counselling represented in terms of function, trust, and expertise; how do genetic counselors discuss genetic counselling in the DTCGT industry? Findings: We identified 21 companies selling genetic tests for psychiatric disorders online. Of these, 15 do not provide genetic counselling (although several provide links to genetic counselling services or recommend genetic counselling). We identified the following types of genetic counselling provision: integrated genetic testing and counselling product; discretionary genetic counselling; genetic counselling for product advice; result interpretation counselling; future health and information strategy counselling. We further consider forms of interaction and information provision (e.g., email, phone); representations of genetic counselling as a profession; and ethical aspects of genetic counselling practice (e.g., privacy, confidentiality). We conclude that both the 'medium' of DTCGT and the 'message' of common genetic polymorphism-based risk assessment have implications for the field of genetic counselling.

P01.48
Informational needs and expectation prior to genetic counselling for hereditary cancer and fulfilment of these needs and expectations

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Background: The fulfilment of counselees' expectations in cancer genetic counselling and how this affects the outcome of counselling have received little attention so far. The aims of the present study was to investigate the informational needs and expectations among counselees prior to their first genetic counselling for hereditary cancer. We investigated also whether these needs and expectations were fulfilled. Furthermore, we explored cancer specific worry, levels of anxiety and levels of depression among the counselees. Lastly, we investigated if there were any relations between fulfilled expectations and levels of anxiety or depression after the counselling.

Methods: One hundred and twenty-eight counselees (response rate 65%) participated in this study. Data were collected both before and after the counselling session. Levels of anxiety and depression were assessed by the Hospital Anxiety and Depression Scale, the level of worry by the Cancer Worry Scale and informational needs and expectations by the Quote-gene ca. .

Result: The majority of counselees were highly satisfied with the extent to which their informational needs and expectations were fulfilled, although some counselees were not satisfied regarding «emotional matters». The mean level of anxiety among the participants decreased after the counselling session. The result also indicates that if needs and expectations were not fulfilled, the counselees reported higher levels of anxiety and depression.

Conclusion: The result reveals that the counselees have high expectation prior to the counselling and these expectations are usually met. In this study,subjects whose expectations were not meet seem to be morevulnerable after the counselling.

P01.49
Recontacting patients: needed, expected or desired? A literature review

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Aim: As our insights and knowledge in medical genetics is continuously expanding, recontacting patients as new information becomes available could be desirable. The purpose of this literature review was to establish the extent to which recontacting patients after previous genetic counseling is needed for, expected or desired by patients and recognised by counselors.

Methods: The starting point for the literature review was a Pubmed search using "Duty to Recontact"[MeSH] and using "recontacting"[All Fields] AND ("patients"[MeSH Terms] OR "patients"[All Fields]) and a search in Web of Science® on topic "RECONTACT OR RECONTACTING". The results were sifted by reading the title and (if available) abstract. The referencelists of relevant papers were also included.

Results: A total number of 22 publications, addressing this issue to some extent, were retrieved. There were few attempts to define the legal, ethical and social context of this issue, mostly based on expert opinion. We found two studies describing surveys among clinical geneticists and genetic counselors and three studies investigating patient's or relative's attitudes. The number of participants was 13-61 patients or relatives/study and 58-252 health care professionals/study. The results of most studies show significant variation in outcomes. A comparison between outcomes of the different studies is hampered by the differences in study population and methods.

Conclusion: We provide an overview on the literature on recontacting patients in medical genetics, indicating that this is a relatively under-researched area. Considering our rapidly growing insights and possibilities in medical genetics, future studies are needed.

P01.50
Congenital and genetic disorders in the Eastern Mediterranean Region: Challenges to public health and priorities to address

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The Eastern Mediterranean Region (EMR) of the World Health Organization (WHO) includes 22 countries in the Middle East and North Africa that are currently facing major challenges in providing comprehensive and up-to-date community genetic services.

This study directed by WHO Eastern Mediterranean Regional Office (EMRO) reviews available data from EMR countries on the current situation of congenital and genetic disorders. Data on the epidemiologic profile and available services was collected from published literature, from questionnaire sheets sent to Ministries of Health, and from personal experience.

Consanguineous marriage is customary in most EMR communities and intra-familial unions currently account for 20-50% of all marriages, with first cousin unions amounting to 20-30% of all marriages.

Haemoglobinopathies in EMR countries show high population carrier rates of 2-7% for ß-thalassaemia , 2-50% for α-thalassaemia, and 0.3-30% for sickle cell, while G6PD deficiency ranges from 2.5 to 27%. In countries including Oman and UAE, birth rate of Down syndrome amounted to 2/1000 births.

Currently, community genetic services such as newborn screening, premarital carrier screening for thalassemias, prenatal screening, genetic counselling, education and birth defects registries are available in some EMR countries. Such genetic services, however, remain patchy and selective.

The basic strategies needed for implementing community genetic services that depend on a combination of basic public health measures, and the education and involvement of the primary health care network will be discussed.

WHO has a crucial role in helping EMR countries planning and

implementing community genetic services and in encouraging regional and international genetic collaborations.

P01.51
A critical appraisal of the private genetic and pharmacogenomic testing environment in Greece

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In the post-genomic era, we are witnessing rapid progress in the identification of the molecular basis of human inherited disorders and the elucidation of genotype-phenotype relationships. The rate of progress has been driven not only by the determination and ongoing decipherment of the human genome sequence but also by the advent of new technological developments that have dramatically reduced the costs of genetic analysis. Consequently, a considerable number of genetic testing centers have emerged, both in Europe and the United States, which offer a plethora of different genetic tests. We have performed a nationwide survey of 18 private genetic testing laboratories in Greece to acquire a better understanding of the genetic testing services that these centers provide, specifically the types of genetic test offered, the target groups, marketing channels, costs of analysis and accreditation. Molecular genetic and cytogenetic testing were found to be the predominant types of genetic testing services offered although there is an increasing demand for pharmacogenomic testing. The main target group for private genetic testing laboratories are physicians who are approached via the Internet, through personal contacts from sales representatives, and at scientific conferences. Genetic testing costs are fairly low in Greece. Although the majority of private genetic laboratories either employ or collaborate with a genetic counselor, few of them are accredited for the provision of genetic testing services. This study provides the basis for a critical appraisal of the private genetic testing environment in Greece and provides a model for replication in other European countries.

P01.52
The impact of genetic testing in reproductive medicine: anxiety and distress three and twelve months after disclosure - an index-control study

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As more and more genetic risk factors for disease are discovered, more genetic tests are offered to patients. None of these tests are properly evaluated for clinical utility, efficacy and psychosocial consequences. In reproductive medicine, couples with recurrent miscarriage (RM) and couples with a man with poor semen quality are karyotyped as part of the diagnostic work-up. We evaluated the psychological impact of receiving a genetic test result in both partners of couples with a genetic abnormality compared to controls.

Methods: Longitudinal prospective index-control study. Participants received 3 questionnaires; just before disclosure of genetic test result, 3 and 12 months after disclosure. Outcome measures were scores on the Becks Depression Inventory (BDI), State-Trait Anxiety Index (STAI) and Impact of Event Scale (IES).

Results: Between 2006-2009, 439 subjects were included. 172 patients from couples with RM (36 indexes, 35 partners, 101 controls), 267 patients from couples with poor semen quality (48 indexes, 42 partners, 177 controls). No differences were found between scores on BDI and STAI between the groups before disclosure or during follow-up. On the IES both indexes and partners had significant higher scores than controls ($p<0.001$). These effects were still present after 1 year follow-up.

Conclusion: Receiving an abnormal genetic test result seems to have negative psychological consequences for both partners of couples with RM or poor semen quality in short and in long term. In the light of ongoing discussions on the efficacy of karyotyping these couples, we need to realise that genetic tests can do harm.

P01.53
Test translation: from a research test to diagnostic test

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New molecular genetic methods provide a vast spectrum of tools for detecting various mutations. Every routine diagnostic laboratory has to evaluate which methods can be adopted from the research setting for routine diagnostics and which are the factors that dictate the applicability of a given method in a diagnostic laboratory. In most laboratories, instrumentation is not a limiting factor, but the challenge is to determine the most cost-effective test, taking into consideration the utility of the test for patients.

We have used Sotos syndrome as a model for test translation. The key issue in successful translation was a close collaboration between academic research and diagnostic laboratory, adding to that a close collaboration with a clinical genetics unit, ensuring the up-to-date knowledge in each phase of translation. We have tested altogether 150 Sotos syndrome samples, and detected a mutation in 30% of the samples. Many clinicians meet Sotos patients rarely and, consequently, the test has been mostly used in cases of suspected Sotos syndrome rather than in confirming the clinical diagnosis. Finding the mutation is valuable for the patient/family, while the value of negative result is less clear and other differential diagnostic diagnoses should be considered. The Sotos syndrome test has been a suitable model for test translation, because the genetic background and genotype-phenotype correlation are well characterised. However, in syndromes where mutational background or genotype-phenotype correlation are less clear, the test for the patients and diagnostic laboratory may remain of low utility, and test translation should be carefully considered.

P01.54
Direct-to-consumer genetic testing for non-health purposes in East Asia

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Background: In 2010, DTC (direct-to-consumer) genetic testing for health purposes have been featured in Europe for its regulation or alert to the public by the ESHG and the HGC in the UK. In contrast, several Shanghai-based companies stated to advertise in Japan to sell DTC genetic tests for non-health purposes, such as tests advertised to identify genes related to an individual's talent, character traits and aptitude. Regulation of DTC genetic tests for non-health purposes have rarely discussed before.

Purpose and methods: To address broad ethical, legal and social implications arising from such tests, we've conducted 1) systematic literature review and policy analysis towards genetic testing in Asian countries including Japan, South Korea, Taiwan and China, 2) questionnaire survey to the Japanese general public to know their recognition and interests towards DTC tests.

Results: Advertisement is commonly observed in East Asia though some of the tests have been banned in South Korea. Our survey shows that 1.3% (47) of respondents has purchased DTC genetic tests, and 72.9% (2,627) of them hasn't recognized such tests. They show higher interests towards tests for health purposes (25.3-53.4%) than non-health purposes (8.2%-21.9%). Younger generation shows higher interests toward DTC genetic tests for their children.

Discussions: The trend of our survey shows the same trend from the survey conducted in 2007. Many parents are deeply concerned about providing education for their children and it's difficult to intervention parental decision to purchase such tests. We should more actively consider issues particularly arisen from such tests.

P01.55
EuroGentest Clinical Utility Gene Cards - guidelines for the clinically validated use of genetic testing

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The Additional Protocol to the Convention of Human Rights and Biomedicine, Concerning Genetic Testing for Health Purpose (Article 6) declares that clinical utility is an essential criterion for deciding to offer a genetic test to a person. Clinical utility refers to the ability of a genetic test to significantly affect the clinical setting and patient outcome. In order to assess this information and make it available to clinicians, geneticists, referrers, service providers and payers, EuroGentest, an EU-funded Network of Excellence, designed the Clinical Utility Gene Cards (CUGCs).

CUGCs are disease-specific guidelines written by international authorships. Based on the ACCE framework (Analytical validity, Clinical validity, Clinical utility, Ethical, legal and social issues) the CUGC backbone consists of three parts: disease characteristics, test characteristics and clinical utility. CUGCs represent concise and comprehensive documents for the clinically validated use of genetic testing. All guidelines are freely available on the websites of EuroGentest, the European Journal of Human Genetics (EJHG) and Orphanet.

As of January 2011, the clinical utility gene cards project receives support by EuroGentest 2 and the European Society of Human Genetics. Initial steps taken for each gene card include the selection of principal authors, the provision of document templates, as well as support during document publication. Subsequently, each CUGC is peer-reviewed and published by the EJHG. From 2011 to 2013, 300 new CUGCs are planned to be established. The regular revisions and inclusion of new developments and findings ensure that all published documents reflect the state-of-the-art.

P01.56
Genetic testing and common disorders in a public health framework - Recommendations of the European Society of Human Genetics

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Currently, the genetics research community is sceptical about the possibilities of genetic susceptibility testing and screening. The Public and Professional Policy Committee of the European Society of Human Genetics, EuroGentest and the Institute for Prospective Technological Studies convened to discuss relevance and possibilities of genetic testing for common disorders in a public health perspective. It is suggested to take the spectrum ranging from monogenic disorders to common complex disorders into account. Associations between genetic variants and disease risk have been established, for instance for hereditary breast and ovarian cancer, colon cancer, diabetes mellitus (MODY subtypes), thrombosis, cardiovascular disorders, celiac disease and Alzheimer disease. Although these examples relate to monogenic sub-forms of common disease, they can nevertheless be used to reflect on possibilities and relevant obstacles in using the new genetics in public health. Recommendations have been formulated stressing: avoiding too high expectations of genomics applications; the need to establish clinical utility of genetic testing before introducing large-scale applications; in case clinical utility is not proven, but likely, research should accompany pilot projects; monogenic conditions can serve as examples for common complex diseases, when identifying etiological pathways and developing healthcare in a responsible way; sufficiently qualified health-care professionals should be involved in case of direct-to-consumer genetic testing; regulation is necessary to guarantee truth-in labelling and truthful promotion of genetic tests; stratified medicine will only be successful if health-care insurance is based on solidarity; especially in developing countries governments should avoid an access gap to genetic testing of proven clinical utility.

P01.57
Genomics education for primary care providers in Estonia

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Since 2000, Estonian Genome Center of University of Tartu (EGCUT), in partnership with primary care providers (PCPs) nationwide, has recruited over 50,000 gene donors for the Estonian Biobank. This collaboration has ensured a collection of high quality health data, as pre-existing medical information has been included. Additionally, it represents a starting point in the working relationship between EGCUT and PCPs, who are expected to facilitate the use of genomic information in the clinical setting once genomic information has sufficient clinical validity and utility. This study investigated the knowledge base and expectations of PCPs. Of 130 PCPs surveyed, 64 responded, representing 8% of the 804 PCPs in Estonia. Overall, PCPs demonstrated eagerness to apply genomic information in practice, as well as a willingness to improve their knowledge base in genetics and genomics. Results also indicated that substantial improvements in genetics education are needed to achieve EGCUT's plans for personalized medicine in Estonia. It is a goal of EGCUT to implement a database that can be accessed by all physicians in Estonia that would contain genomic data, along with all other medically relevant information on the patient, and could be used in medical counseling for and effective application of genomic information in clinical care. Several additional factors in Estonia may help to realize that goal: the national health care system, availability of electronic health care records, and the fact that University of Tartu is responsible for the education of health care workers as the only medical school in the country.

P01.58
One more proof that practice makes perfect

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The aim of this study is finding a way to determine better achievements in learning genetics. Motivation for a more thorough learning at the end of the first semester is a final examination, although many students bear in mind the wish to practice in another field beside medical genetics. Because "practice makes perfect" in every field under the proper guidance, I have tried to discover if repeating the information could change the marks students obtained.

The study group consisted of 128 first year medical students, who were tested twice. During the middle of the term they received marks for answering two questions: one from the practicals' and one from the lectures' content. Then all the questions from the lectures were discussed, so students could learn what was expected as an answer. Despite lack of time, these answers were briefly repeated at the beginning of the next practical. To view the impact of repetition, I compared these marks in each of the two questions with those obtained at the end of the term. At this final test 88 students had to answer exactly the same two questions as the first time, while 30 control students received different ones, randomly distributed, but from the same question pool.

In both groups about 70% of students had better results at the second testing, supporting consequently the idea that when teaching medical genetics one can mould his activity, repetition providing a better and therefore more satisfying outcome for both trainer and trainee.

P01.59
Hearing impairment data analysis in clients of the genetic counseling network of Iran welfare organization (1999 - 2009)

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The importance of genetic and congenital factors which causes many diseases and disabilities is obvious over the world.In Iran,Prevention of Genetic Disabilities program has been planned with the aim of implementation of a genetic counseling network.For implementing this program The network has gradually expanded since the year 1997,now we have 169 genetic counseling centers throughout the country. One of the chief complaints of clients of these centers has been the history of hearing impairment.In this study we have gathered the data from 1999 until 2009 about hearing impairment in our provincial referral centers in genetic counseling network.The total number of files having deafness history in pedigrees among 118786 files are 6521(5.5%).The total number of files having deafness history in each of spouses is 960(14.7%),in both spouses is 625(9.6%), in offsprings is 1460(22.4%),in one Offspring is 957(14.7%)&in two or more offsprings is 503(7.7%).The total number of files which are positive for Connexin 26 is 168(2.58%),Connexin 26 & 30 is 8(0.12%),other nonsyndromic AR genes is 7(0.11%),other syndromic AR genes is 26(0.4%),nonsyndromic AD genes is 1(0.015%) and syndromic AD genes is 5(0.08).The total number of deaf people is 9817: 5313(54%) males and 4512(46%) females.The numbers of different types of consanguinity among the clients (the total number of files with deafness history) are double cousins: 87(1.33%), first cousins:2803(42.98%),first cousins once removed:310(4.75%),second cousins:720(11.04)&nonc onsanguineous: 2839(43.54%).The total number of files with deafness history due to different modes of inheritance in pedigrees are: environmental factors:87(1.33%), nonsyndromic AR:2961(45.41%), syndromic AR:269(4.13%), nonsyndromicAD:400(6.13%), syndromic AD:70(1.07%), nonsyndromic X-Linked:32(0.49%), syndromic X-Linked:9(0.14%), mitochondrial:3(0.05%) & unknown causes:1171 (17.96%).

P01.60

Are genetic testing technologies driving clinical practice?

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As the demand for genetic analysis is increasing in the health care system, the extension of diagnostic tests for genetic disorders is needed. The majority of genetic diseases are molecularly and clinically highly heterogeneous and until recently, the available techniques lacked the required capacity to test many genes in parallel. Next generation sequencing technologies already available in research provide a unique opportunity to develop new diagnostic tools for heterogeneous genetic diseases. But their clinical use raises major and previously unaddressed issues. In a context of innovation, implementing new technologies is aiming to optimize clinical care. How to preserve the essence of traditional clinical values and how to maximize the translational relevance of genetic research for patients and for society? A main concern is that the rapid penetration of systematic technologies into genetic medical departments blurs established frontiers between research and clinics. A huge amount of personal medical data will indeed be produced, including results irrelevant to any particular clinical problem but which may be of importance to the patient in other ways or in the future. Our present inability to interpret most of the data requires ethical consideration. Questions of personal data storage, possible updating and conditions to re-contact patients and families require the development of appropriate rules or guidelines prior to clinical implementation. Clinicians, public health practitioners and relevant decision-makers should also determine their strategies and approaches in an era of such rapid advances. Main general challenges raised by High Throughput Technologies will be discussed and some positions proposed.

P01.61

Genetic testing for Lynch syndrome: Psychological impact and opinion about reproductive decision-making among mutation carriers

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Background: New assisted-reproduction techniques as prenatal diagnosis (PND) and preimplantation genetic diagnosis (PGD) are sometimes proposed in inherited cancer predispositions. These techniques are debated in some European countries regarding Hereditary NonPolyposis Colorectal Cancer (HNPCC). This interview study aimed to assess the psychological impact and the opinion about reproductive decision-making in HNPCC mutated individuals, after genetic test results disclosure.

Methods: A self-report questionnaire including socio-demographic data, psychological status and reproductive options was retrospectively sent to 101 HNPCC mutation carriers (patients and their relatives) from a complete oncogenetic colorectal data-base between 1997 and 2010. The prevalence of posttraumatic stress disorder (PTSD) was assessed at the time of result disclosure and at least one year later, using IES-R(Impact of Event Scale-Revised)

Results: Complete data were available for 34 individuals (25 relatives, 9 patients): 17 males, 17 females, median age of 33.5(22-59), with mutations in hMLH1 (15) hMSH2 (15) and hMSH6 (4) genes.

Only 13.8% of participants reported significant immediate PTSD. They mainly explained their distress by fear of premature death (43.3%) and passing on mutated genes (41.9%). For 90.3% of respondents, reproductive decision-making remained unchanged after results. Among reproductive options, PND/PGD or spontaneous natural conception were equally reported (50%). None of socio-demographic or psycho-medical variables were significantly associated with both choices.

Conclusion: A minority of mutation carriers reported significant levels of distress after result genetic disclosure. Half of respondents were in favour of PND/PGD (as much as those interested by natural conception) but we are taking about an opinion and not their intention.

P01.62

Oncogenetics as a tool of cancer personalized medicine: a two-year experience in a public hospital in Brazil

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The purpose of Oncogenetics is twofold: to identify a portion of population with increased risk for cancer by identifying those with significant family history and to successfully intervene in these individuals reducing their incidence of and mortality from cancer by enhanced and directed genetic screening, early detection, prophylactic surgery, and so forth.

This individualized approach has been carried out for the last two years in Hospital Federal de Bonsucesso, the biggest and most important general hospital in Rio de Janeiro, performing almost 500 oncology consultations monthly and about 600 cancer surgeries yearly . As about 10% of these cases are familial and non-related, there are 60 new cancer-prone families undergoing our attention yearly. With regards to colorectal cancer, 20 HNPCC families and 3 FAP patients have been identified based on family history and pathology data, being referred to genetic counseling and specific cancer control strategies. As to less frequent conditions, patients with VHL Disease, Muir-Torre Syndrome and Nevoid Basal Cell Carcinoma Syndrome have been identified being under close follow-up and individualized genetic counseling.

In conclusion, the experience of our hospital shows that clinical geneticists working in close contact with oncologists and surgeons are of utmost importance for directing clinical investigation, especially in places where more specific examinations may be unavailable. Oncogenetics represents a true bridge closing the gap between basic sciences and clinical medicine, and can surely play a pivotal role in any interdisciplinary team devoted to cancer care and willing to provide a comprehensive approach to their families.

P01.63

Action research of genetic education on human diversity targeting mothers of pre-school children in Japan

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<Background> Japan is basically a homogeneous society in culture and population, therefore people are less aware of human diversity. We are intending to perform the action research of the genetic education to mothers of pre-school children. At first, it was necessary to know the participants' basic knowledge of genetics where understanding of human diversity is most preferable subject to discuss.

<Methods> We recruited 8 mothers participating in the University affiliated kindergarten who accepted the study and were willing to discuss a theme: "*everybody is different therefore everybody is valuable*". Throughout the Class and Discussion at eight times, the discussion was qualitatively analyzed. The study protocol was approved by the ethical committee of the Tokai University.

<Result> Three concerns were raised from discussion on the theme: "troublesome with surroundings", "inherent lack of individual ability", and "discrimination to disease/disablity". After the discussions, the participants felt the self-contradiction and genetic knowledge is needed for better understanding of human diversity.

<Discussion>The participants had conflicted thought to the human diversity, and were willing to know more about human diversity on the course of the discussion. In conclusion, importance of genetic education to general population for the society is confirmed.

P01.64

Psychological impact of presymptomatic DNA testing on Huntington's Disease gene carriers

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Aim: The present study is finalized to explore the psychological and "quality of life" impact of genetic testing on presymptomatic Huntington's disease (HD) gene carriers. This is a part of a larger research and has only a descriptive and preliminary purpose.

Methods: From May 2009 to October 2010 we evaluated 18 presymthomatic subjects resulted carriers of HD gene, 6-12 months after the genetic testing. The instruments used are the following: 1) a semi-structured interview; 2) the Psychological General Well-being Index (PWBGI); 3) the Short Form 36 Health Survey Questionnaire (SF-36).

Results: Eighteen patients (M:5, F: 13; mean age: 48,52, range age:28-75) were assessed.

The means and standard deviations of the PWBGI scales are: Anxiety 16,38 (4,90), Depression 11,55 (2,57), Well-being 10,66 (3,56), Self control 10,56 (3,53), Global health 10,44 (4,55), Vitality 11,78 (4,18).

The means and standard deviations of the SF-36 scales are: Physical functioning 78,61 (30,83), Role-Physical 62,5 (50,62), Bodily pain 82,28 (30,13), General health 51,83 (31,66), Vitality 54,72 (25,34), Social functioning 72,92 (28,75), Role-emotional 70,39 (51,64), Mental health 60,67 (18,98). Sample mean scores were found lower than the normative scores.

Conclusions: The current preliminary findings show a critical impact of DNA testing on psychological well being and the quality of life of the presympotamic HD gene carriers: they were characterized by lower physical functioning, less vitality and social support, higher level of anxiety and depression. The data also suggest the importance of a specific psychological intervention. Further research with a larger sample is needed.

P01.65

Hypermobility Syndrome (Ehlers-Danlos Syndrome Hypermobility Type) in Japan

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Background: Hypermobility Syndrome (HMS, ICD-10 M357) is a connective tissue disorder characterized by generalized joint hypermobility and chronic pain (major criteria in Brighton criteria (2000)). Both the pathogenesis and the causative gene are not known. Even healthcare professionals are not normally familiar with HMS patients. We have developed a draft diagnostic criteria for HMS in Japan based on our past survey for clinical manifestations in our twenty patients and worldwide previous studies.

Method: With our draft diagnostic criteria and a summary of the condition from GeneReviews, we have sent 455 departments in university hospitals and orthopedic hospitals a survey form investigating whether they have any experience with patients with possible diagnosis of HMS. The departments included orthopedics, anesthesias, rheumatics and genetics. Our draft diagnostic criteria is as follows: Essential items 1. Generalized joint laxity (a Beighton Score of 4 or more), 2. Recurrent dislocations/subluxations in multiple joints without known/obvious trauma, 3. Chronic pain which persist more than 3 months. Notices 1. Skin hyperextensibility/loose skin is not necessarily seen in HMS patients, 2. Those who are already diagnosed with EDS will be included, 3. Joint laxities may recur after operation to stabilize the joint had done, 4. Other symptomatic diseases such as chromosomal abnormality should be excluded.

Result: 207 forms were collected (45%), 27 departments answered they have experienced HMS patients in the past, the total number of patients reported was 77 (47 if limited within past one year). Secondary survey is currently in progress.

P01.66

Attitudes toward termination of pregnancy based on prenatally detected genetic disease or malformations in selected groups of doctors-in-training

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Background: This study explores attitudes towards termination of pregnancy (TOP) in different scenarios between different groups of doctors-in-training working with TOP.

Danish law permits TOP on request of the woman until gestational age (GA) 11+6 and TOP by permission after application to a local abortion council until approximately GA 22+0.

Methods: Doctors-in-training aiming for specialisation in obstetrics and gynaecology (OG-i-T) or clinical genetics (CG-i-T) completed a questionnaire regarding their views on TOP in different scenarios.

A pro-abortion-index was derived from their responses.

Results: 96 doctors-in-training completed the questionnaire. Of these 77% aimed for specialisation in obstetrics and gynaecology, 20% aimed for clinical genetics and 3% were undecided. Mean number of years since graduation was 7.0; mean number of months in the specialty of choice was 25. The pro-abortion-index was normally distributed (mean 10.1, SD 3.2). CG-i-T were more pro-abortion as a group than OG-i-T (p = 0,02).
Results are summarised in table 1.
Discussion: Doctors-in-training were more acceptant of TOP based on genetic disease and foetal abnormalities than TOP for psychosocial reasons. A similar tendency has been reported for nurses and medical students. The responses for upper GA-limit varied with the specific diagnosis. Both groups favoured abortion on request longer than Danish law currently permits. Both groups agreed with current practice for late terminations.
Table 1

Scenario		GA in weeks (95% CI) until which participants thought that TOP should be possible		
		OBGYN-in-training	Clinical geneticists-in-training	P-value OG-i-T vs CG-i-T
Psychosocial problems	On request	13.1 (12.5-13.7)	15.3 (13.6-16.9)	0.003
	With permission from abortion council	19.9 (19.2-20.5)	19.8 (18.2-21.5)	0.84
Foetus with Down syndrome	On request	16.5 (15.2-17.8)	17.9 (16.2-19.5)	0.28
	With permission from abortion council	22.0 (21.2-22.8)	22.4 (21.7-23.1)	0.62
Foetus with cystic fibrosis	On request	15.8 (14.8-16.9)	16.9 (15.1-18.8)	0.31
	With permission from abortion council	21.3 (20.7-21.9)	21.2 (19.8-22.0)	0.88
Foetus with polycystic kidney disease	On request	14.1 (13.2-15.1)	15.7 (14.0-17.3)	0.12
	With permission from abortion council	20.0 (19.2-21.0)	19.2 (17.2-21.1)	0.35
Minor malformation (limb defect) detected with routine ultrasound	On request	13.8 (12.9-14.7)	15.4 (13.7-17.1)	0.09
	With permission from abortion council	17.5 (16.3-18.7)	18.9 (16.7-21.1)	0.27
Pro-abortion-index		9.7 (8.9-10.5)	11.7 (10.4-12.9)	0.02

P01.67
Neonatal screening for hemoglobinopathies in the City of São Carlos, São Paulo, Brazil: an analysis

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The neonatal screening is the major Brazilian Unified National Health System (BUNHS)'s initiative in medical genetic's area and it embraces four diseases: inborn hypothyroidism, phenylketonuria, hemoglobinopathies and cystic fibrosis. This project intends to verify the National Neonatal Screening Program (NNSP)'s effectivity and to estimate the hemoglobinopathies' incidence in the City of São Carlos, São Paulo, Brazil. It concerns a descriptive study, undertaken between 2007 and 2010, when 11,818 children were born, of which 10,589 (93.55%) were subjected to the BUNHS's screening. The screening's results showed 360 abnormalities (3.39%), with an incidence of 1.85% falciform trace, 0.009% sickle-cell disease, 0.45% C trace, 0.37% alpha-thalassemia, 0.03% suspected beta-thalassemia, 0.07% low fetal hemoglobin and 0.14% undetermined hemoglobin, mean, a hemoglobin variant not identified by test. There was 1 (0.009%) falciform trace associated with Bart's hemoglobin and 2 (0.018%) C trace associated with Bart's hemoglobin. In 48 cases (0.45%) the results were inconclusive. Although the PNTN's reasonable cover, the numbers of inconclusive results and undetermined hemoglobins are considered high yet, as well as the newborn's percentage with low fetal hemoglobin, giving an indirect idea of the percentage of children who took the screening after 21 days of life, when the fetal hemoglobin is replaced by the adult hemoglobin. The results points to the need for a consolidation of a line of comprehensive health care for patients with hemoglobinopathies and their family in the City of São Carlos.

P01.68
Providing genetic risk information to parents of newborns with sickle cell trait: role of the general practitioner in neonatal screening

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Purpose: In 2007 the neonatal screening program in the Netherlands was expanded, now including sickle cell disease (SCD). SCD carriers are also identified. The benefit of reporting SCD carrier ship includes detection of high risk couples (both parents are carriers) who can be informed about future reproductive choices, a responsibility of their general practitioner (GP). We evaluated knowledge, ideas and reported actions of GP's about SCD carrier ship and explored reported potential barriers.
Methods: A questionnaire study.
Results: 139 GP's responded to our questionnaire (49%). 90 GP's (90%) stated they informed parents of the test result. In only 23 cases (23%) both parents had themselves tested for hemoglobinopathies. 81 GP's (64%) stated they did not have enough clinical experience with SCD. Almost half of the GP's indicated they did not experience any barriers in counseling patients (n=60, 48%).
Conclusion: At the moment the goal of the neonatal screening for SCD carrier ship has not been achieved as the majority of parents were not tested for hemoglobinopathies after disclosure of carrier ship in their newborn. With GP's reporting few barriers in counseling parents and only indicating a lack of knowledge and clinical experience, more effort is required to provide better information to GP's to help facilitate their work.

P01.69
Parents' opinions on storage of heel-prick cards for research purposes

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This paper reports on a recent quantitative survey of over 1600 Dutch parents' views about the storage, management, and secondary use of heel-prick cards. Given the scale of neonatal screening, these blood samples present a wealth of biological data for medical research. While not a conventional biobank, the card collection could be treated as such if there would be societal support and a (new) management body and use policies could be developed. There is currently a discussion in the Netherlands about prolonged storage, independent management and extended secondary uses. Our survey was aimed at parents of children aged 0-5 whose heel-prick cards are presently in storage, as well as parents-to-be. The survey quantifies for the first time the views of this group about current and future options related to neonatal screening, permission for use in medical research, and issues related

to (length of) storage, management and secondary uses of the cards, including medical research, diagnostics, and identification. The survey contains questions on knowledge of current storage, management and use policies (6 items), personal experience with heel-prick testing (4 items), demographic information (9 items), and opinions on specific storage, management, and use issues (41 items). Respondents were able to indicate their level of support for 10 categories of secondary use, including "to determine how often certain medical conditions occur in the Netherlands" (prevalence studies) and "to find out how certain diseases develop" (etiology studies). Respondents were also asked how long the collection of heel-prick cards should be kept for medical research.

P01.70
Patient and public involvement in genetic and developmental medicine research

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The Manchester Biomedical Research Centre (MBRC) specialises in genetics and developmental medicine, aiming to transform healthcare through translational research. Patients and the public are at the centre of the MBRC effort to move developments from the laboratory into clinics.
Patient and public involvement (PPI) in healthcare research has been shown to increase its relevance and effectiveness. A recent mapping exercise carried out at the MBRC showed a range of PPI activity taking place. This paper highlights three examples of good practice.
(1) A patient advisory group (PAG) was formed to help design, translate and disseminate the outcomes from a programme of research looking at improving services for patients with inherited retinal disease. The PAG is made up of patients, representatives from a range of charities for visual impairment and Genetic Alliance and is attended by the wider study team, including the primary investigator, health care researchers, genetic counsellor and health economist.
(2) A parent of an affected child is a partner on the Dyscerne guidelines group, whose aim is to develop and disseminate clinical management guidelines for rare dysmorphic conditions. This has resulted in the inclusion of important information aimed at professionals dealing with children who have rare syndromes.
(3) A public consultation was carried out with women at the surgery of their general practitioner. Women were asked to feedback their views of both cervical screening and proposed interventions to increase uptake via a questionnaire. The consultation increased confidence in the proposal and provided support for the study trial design.

P01.71
Newborn screening for Pompe disease? Exploring professional views

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New developments in enzyme replacement therapy have kindled discussions on adding Pompe disease to neonatal screening programmes. Traditionally, expanding screening is being discussed among a restricted number of medical experts. In this research we aim to explore the views of a wide range of relevant professionals to gain more insight into the process of weighing pros and cons of neonatal screening for Pompe disease.
We conducted semi-structured interviews with over 25 professionals in the Netherlands. Some had experience with Pompe disease patients, such as paediatricians, neurologists, physiatrists, family doctors and patient organisation representatives. Others were involved in the organisation of screening or health care, such as representatives from the Centre for Population Screening, the Health Care Insurance Board, and well-baby clinic doctors. They were asked about their first reaction to neonatal screening for Pompe disease, after which benefits and harms and requirements for screening were explored in more detail.
This study shows that many professionals expect benefits from neonatal screening for Pompe disease, especially for early-onset cases, since it has been shown that prompt treatment improves survival and health status in many affected infants. A distinctive feature of this type of screening is the detection of late-onset cases that would otherwise present anytime between infancy and later in adulthood. Whereas some interviewees considered early detection a benefit in these cases as well, allowing for monitoring and timely treatment, others considered this a drawback or a reason not to screen, given the psychological burden.

P01.72**
Governance and future of genetic preconception carrier screening

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Recessive disorders cause serious morbidity and mortality in 25/10,000 children. Most of them are born unexpectedly without family history of the condition. In about 1/100 couples, both partners are carriers of the same disorder, facing 25% risk of affected offspring in each pregnancy. Carriers are usually unaware of their carrier status.
Preconceptional carrier screening enables carrier couples to make informed reproductive decisions before pregnancy. Our offers of preconceptional carrier screening for cystic fibrosis (CF) and/or hemoglobinopathies (2002 and 2005) were favoured by the target population and stakeholders. Despite positive results in pilot studies, meeting genetic screening criteria and constructive debates about ethical, technical and financial aspects, in most European countries a systematic healthcare offer of preconceptional carrier screening for the general population is lacking. Meanwhile, uncontrolled commercial private testing is available online. Is this development desirable in terms of informed consent, counselling, and medical supervision? In the absence of a healthcare offer, the Clinical Genetics Department of VU University Medical Center in Amsterdam recently started to offer preconceptional CF screening tests on the hospital website, accompanied by sufficient medical information, without commercial goals and with availability of counselling. The experiences of (potential) users will be monitored and integrated when further developing the service. Is this the ideal answer in diversifying contexts of screening? Will screening for more recessive disorders become available in regular healthcare in the future? And how to enable beneficial application of whole genome sequencing, which allows parallel screening for all known recessive disorders, in this preconception setting?

P01.73
Effects of predictive genetic testing for cardiovascular risk in healthy overweight subjects.

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Aims: To compare effectiveness of common medical recommendations to reduce the weight as it is a confirmed risk factor for future cardiovascular pathology in a group of overweight healthy young subjects with the counseling supplemented by genetic test for cardiovascular disease predisposition. Materials & methods: two groups were collected in a similar way: group 1 (control) (n=95, average age 31 years, body mass 84.9 kg) got recommendations that they are overweight and have to reduce their body mass in order to lower the risk of future CV pathology; group 2 (experimental) (n=103, average age 30.8 years, body mass 89.0 kg) got similar recommendations and additionally got results of a genetic test assessing individual risk of CVD. Both groups were examined after a year and body mass was measured repeatedly. Results: Both groups reduced their weight significantly. Control group lost 1.51 kg ($p<0.0001$), while experimental - 2.29 kg ($p<0.0001$). The weight lost did not differ statistically between

two groups. Analyzing separately subgroups of men and women showed that men lost their weigh independently from the fact of getting personal genetic cardiovascular risk estimates (1.5 kg and 1.8 kg in groups 1 and 2 accordingly), while women reduced their weight mainly in the group with genetic testing - 4.2 kg compared to control group who lost 0.8 kg. Conclusions: Our data reports that there can be gender-specific reaction to genetic testing. It was shown in previous studies that women in our population have more positive attitudes and beliefs to predictive genetic testing.

P01.74
Ethical Issues in pre-implantation genetic diagnosis in Portugal: a comparative analysis of professional's opinions in years 2000 and 2010 using questionnaires

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Medical genetics has made significant progress in the last decades, especially in the field of prenatal testing. After the dramatic expansion of prenatal diagnosis that started in the seventies, pre-implantation genetic diagnosis (PGD) became a reality in 1990, following advances in the techniques of medically assisted reproduction (MAR). Ethical problems related to this technique start well before the analysis: it is necessary to offer appropriate genetic counselling, to obtain informed consent for the necessary procedures and to maintain strict confidentiality of the whole process. The main ethical problems concern the status of the embryo, the investigation and the manipulation of embryos, eugenic or sex selection and the provision of resources. In 2000 a questionnaire addressing several of the principal ethical concerns, namely the attitude towards PGD, embryos and genetic testing, was distributed to the Directors of the five largest MAR centres in Portugal; in 2010 the same questionnaire was sent to 27 MAR centres and answered by 11. In all cases it was required that the answers should be based on the general policy of each centre. This work presents the comparative analysis of all the obtained data, particularly focusing on the main ethical problems related to this diagnosis, i.e., the status of the human embryo and the attitude of the genetic professionals working in this still relatively new, and very specific, field of genetic diagnosis.

P01.75
Prenatal diagnosis based on informed choice

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In general, prenatal test is recognized as population screening in many countries. For example, more than 80% women have maternal serum test as screening in the UK. In Japan, we do not offer maternal serum test as screening in most obstetrics services. We might provide the information of screening test and further chromosomal test such as CVS and amniocentesis with the information of genetic counselling. There is no regulation for prenatal diagnosis in ultrasound diagnosis, so some clinic would offer screening at some point and some do not. We do not scan the fetus as screening without patient's request at our hospital.

Historically, we have been concerned with prenatal diagnosis over 30 years at our hospital. At first, we had been doing only the test and given the result for referred patients; however we now offer genetic counselling for referred patients to avoid automatic screening and to have informed choice for prenatal diagnosis.

Prenatal diagnosis contains ethical issues all the time, but most pregnant women do not notice this problem until they consider termination at some point. Genetic counselling in prenatal diagnosis could be good support for the patient who has to face difficult ethical issues during pregnancy. It sometimes reduces unnecessary risk for worries and/or invasive test.

P01.76
Information related to prenatal genetic counseling: Interpretation by teenage students and ethical implications

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Among current teenagers we find a large proportion of future parents, some of whom will be seeking prenatal genetic counseling. Getting raised in the genomic era may not only increase the knowledge of available genetic testing but may also have an impact on how genetic information is perceived. However, little is known about how this teenage group reacts to the language commonly used by health care professionals providing prenatal counseling. In addition, as risk communication is related to numbers and figures, having different educational backgrounds may be associated with separate risk perceptions. In order to investigate these issues, a previously developed questionnaire (Abramsky & Fletcher, 2002) was administered to high-school students in Sweden. A total of 344 questionnaires were completed by students belonging to a natural science or a social science program. Our data show that teenage participants were particularly worried by the use of technical jargon and words like *rare* and *abnormal*. Negative framing effects and perception differences related to numeric risk formats were also present. There were some cases of gender and educational program effects on risk assessment but this outcome was not generalizable. Besides the questionnaire results, we discuss the ethical implications of the data based on the norm of non-directiveness and try to provide some basic guidelines. In general, genetic counselors should be aware that the language used within clinical services can be influential on this group of upcoming counselees.

P01.77
From rationing to rationality: an n-of-one trial service for off-label medicines for rare (neuromuscular) diseases

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Background: In the Netherlands, expensive medicines are not reimbursed for off-label use without sufficient evidence of efficacy. Patients with rare diseases are disadvantaged because the burden of proof is difficult to meet. There are obstacles both for industry and academia to performing large-scale randomized, controlled trials. Moreover, reimbursement rules discourage doctors from prescribing expensive medicines off-label, even to small groups of patients. Examples of reimbursement problems with off-label medicines are known from many rare disease areas, including genetic disease. Controlled n-of-one (single-patient) trials with internal randomisation (e.g. AB-BA-BA) could generate new evidence for rare, chronic conditions where the aim of treatment is symptom control.

Objective: This project aims to initiate development of an n-of-one trial service, embedded in the Dutch health care system. Pilot implementation will be designed, focusing on neuromuscular diseases as a model.

Methods: Reimbursement problems with off-label medicines for rare neuromuscular diseases are being inventoried among neuromuscular specialists and patients with neuromuscular disease in the Netherlands. A consensus meeting will be organized to define legal, ethical and scientific preconditions for formalizing and sustaining an n-of-one trial service. An electronic data registry system for n-of-one trials will be designed, and several protocols will be written for specific trials to be performed in a pilot implementation of the n-of-one trial service.

Implications: If an n-of-one trial service is successfully designed, a subsequent project can pilot trials for off-label neuromuscular indications and eventually others. Societal acceptance of n-of-one evidence may also stimulate development of genomic therapies tailored to rare genotypes.

P01.78
Volunteers involved in working with NGOs for rare diseases

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In the absence of a national strategy for rare diseases (RD), in the last 20 years - the most neglected group of diseases in Romania - the information and social inclusion of these children remains to the initiative of NGO's. The start was in 2006 and working with volunteers was for the first moment, the solution. Aim of our work was to focus on the changes in life quality of children with these diseases, after teamwork and a multidisciplinary approach. Material and methods. The volunteering activity intend to contribute, both by involving as many volunteers as possible and by providing information, coming directly into contact with patients and their families, change their perception and in future the civil society perception towards the rare diseases' issue. Main activity was based on specialized volunteer-patient- family direct relationship, making use of playing games, art and group therapy in weekly meetings. Results. Every year since 2008, volunteers *Save the children* and ANBRARo have organized "Rare Diseases Day" by handing out leaflets to the people, for a week, in a crowded square from Timisoara and other Romanian cities. These informing campaigns were very well received by the passer- byes, media and people with (RD). Being medical students and in the company of children with RD, volunteers were able to participate at conferences and other meetings with scientific papers. They also provide active help to the children so that, the parents could stay and learn more about their children's disease in meetings dedicated to support groups.

P01.79
Orphanet UK 2011: More information, more specific and easier to retrieve

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Orphanet is the largest international online portal for rare diseases and orphan drugs. Orphanet lists services for around 6,000 rare diseases in 36 countries. Among these services there are over 21,000 medical laboratory tests, 1,200 clinical trials, 1,000 registries and 4,900 expert centres.

Information regarding the UK has grown strongly over the last two years. Today there are over 70% more clinical trials, 45% more registries and biobanks, over 50% more medical diagnostic tests and 30 % more clinics.

Relevant information is highlighted and retrievable; from over 170 clinical trials available, 80% are ongoing and over 50% are recruiting participants. There are also over 80 registries and biobanks from which 25% are recruiting. Laboratories with accreditations are highlighted. Designated centres of expertise display the Reference Centre label.

All these services can be searched by disease or by gene. Moreover, research activities can also be retrieved by type, by institution/laboratory, by professionals, by sponsor/funding body and by substance/tradename (clinical trials). Diagnostic test can be filtered by city or type of accreditation and clinics by type of service (medical management/genetic counselling and paediatric/adult clinic).

The webpage has also improved its user friendliness with the addition of two new features: the "google translational tool" that allows the user to translate any page in different languages and the "autocompletion tool" that facilitates research by providing suggestions as terms are entered. Moreover, a new website for the UK has been created to offer national relevant information for patient and professionals.

P01.80
The "DICE-APER Registry": a new online tool to attend the patients with Rare Diseases in Primary Care.

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Our purpose was to design an online Registry in order to manage patients who suffer a Rare Disease (RD) in Primary Care (PC). This Registry might be a quick and useful tool for General Practitioners. It could help to follow-up the RD patients and their families in PC. Also to improve its National Spanish Registry.

The Workgroup of "Clinical Genetics and Rare Diseases" of SEMFYC in collaboration with the Institute of Research of Rare Diseases of the Institute of Health Carlos III, the Spanish Federation of Patients with RD (FEDER), and its families (CREER), has designed the online Registry "DICE-APER": http://dice-aper.semfyc.es

The PC is the "gateway" to access to the National Health System and most of the PC units have connection to Internet. Both resources have been used to design an online tool to manage "forgotten" or unknown aspects in the daily practice. As result we have an online tool that: 1) allows to identify every RD, 2) invites each patient to participate in the Biobank at the Spanish National Registry of RD, 3) coordinates genetic counselling, 4) offers health care and social resources 5) and encourages working with other Medical Specialities.

This online tool will help on growing the Spanish National Record of RD and, therefore, we might hope an increasement of the resources that are focused to these diseases in order to obtain the satisfaction of patients. It's important to create and to use new online tools to improve several aspects of the RD.

P01.81
Pre-marriage Screening Program for Beta-Thalassemia in Isfahan (center of Iran) (2005-2010)

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Thalassemia is the most widespread single gene disease in Iran. This report presents the results of pre-marriage screening program applied in Isfahan province (center of Iran) within 5 last years (2005 -2010).

The information related to 22 screening and genetic counseling centers was collected throughout the province within this period. Data analysis was performed by excel soft ware.

Overall 233400 couples were screened from March 2005 till March 2010. Totally 86352 of couples (3.7%) were anemic. In 4.6% of anemic couples Hb A2 was more than 3.5% and they were confirmed for beta-thalassemia trait in the first stage. Overall 61% of anemic couples were undergone iron-therapy that it was in range of 30% in early years to more than 70% in the last one which genetic counseling centers had been developed. Also CBC indices were modified in 69.1% of these couples after treatment for 3 months. Finally genetic testing was performed in 94% of the couples who had not been treated and beta-thalassemia trait was ruled out in 76% of them. We concluded that genetic counseling and iron-therapy has a significant role to reduce under-care suspected couples (false suspected), so that if it is invigorated the surveillance system will be concentrated to care of real carriers.

P01.82
Pharmacogenetic Distribution of Warfarin and its Clinical Significance in Korean Patients during Initial Anticoagulation Therapy

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During warfarin treatment, determining the optimal dose and maintaining the target PT-INR are challenging. Increasing evidence supports the theory that genotypic polymorphisms influence an individual's warfarin dose requirement. In this study, we evaluated allele frequencies and effects of *CYP2C9* and *VKORC1* on warfarin response during initial anticoagulation therapy in Korean patients. We enrolled patients who had initiated warfarin therapy and undergone PT-INR testing at least three times within the first month of anticoagulation therapy. All the participating patients were tested for the detection of *CYP2C9**3 (c.1075A>C) and *VKORC1*-1639G>A. A melting-curve analysis after

real-time PCR was performed using CYP2C9˚3 and VK1639 genotyping kits (Idaho Technology, USA). A total of 37 patients were enrolled in this study. *CYP2C9**1/*1 (86.5%) and *VKORC1*-1639AA genotypes (89.2%) were predominant in Korea. The *CYP2C9**3 and *VKORC1*-1639G alleles were found in five (13.5%) and four patients (10.8%), respectively. Patients with the *VKORC1*-1639G allele received a higher warfarin dose (*P*=0.002), with a significant dose change between days 1 and 30 (*P*=0.003). Patients with the *CYP2C9**3 allele tended to show a more rapid PT-INR increase and receive a lower warfarin dose, with a greater dose change between days 1 and 30. The *CYP2C9**3 and *VKORC1*-1639G alleles influenced warfarin response during the first month of anticoagulation therapy. Considering *CYP2C9* and *VKORC1* genotypes when estimating warfarin dose and frequency of PT-INR monitoring can improve therapeutic outcomes with fewer complications.

P01.83
DNA degradation analysis in post-mortem soft muscle tissues in relation to accumulated degree-days (ADD)

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After the death of an organism DNA starts to degrade: as the organism's cell structure breaks down, nucleases are released and directly cause DNA degradation. Subsequent colonisation, action of insects and microorganisms also contributes to the DNA degradation. As the post-mortem interval (PMI) increases, DNA continues to degrade until no high molecular weight DNA (HMW-DNA) remains.

In order to assess DNA degradation in the model organisms chosen (pig and rabbit), two nuclear genes, Connexin 43 and RAG-1, were aligned to identify conserved regions. Primers were designed to amplify 70 bp, 194 bp, 305 bp and 384 bp amplicons. The primers were also designed to amplify human DNA, which allowed the use of commercially purchased DNA standards to be used as controls. Following DNA extraction PCR analysis was performed using the four primers sets in a multiplex (4-plex): the PCR was optimised so that it worked over a wide range of template amounts (0.1 ng to 75.83 ng). The multiplex (4-plex) PCR was found to work efficiently in triplicate samples of all three species down to 0.3 ng of DNA template.

This multiplex has been used to assess whether DNA degradation can be predicted by accumulated degree-days (ADD), which provides a measure of both time and temperature.

Full 4-plex profiles were generated until day 7 (ADD 111.93) from whole carcasses and body fragments and up to day 11 (ADD 172.48) from insect-activity-free muscle samples.

Future work will include; development of real-time PCR quantification assays, DNA Fragment analysis and DNA preservation.

P01.84
Attitudes of midwives and family physicians towards ethnicity based haemoglobinopathy carrier screening: exploring current behaviour and future intentions.

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Purpose: Globally approximately 5% of the world population is a carrier of a serious haemoglobin disorder. No formal recommendations concerning preconception or antenatal testing of carrier status exist in The Netherlands at present. Earlier research identified registration of ethnicity as an issue in deciding not to introduce a screening program in the past and found that health practitioners did not perceive ethnicity as an increased risk factor.

This study investigates attitudes, behaviour and intentions of midwives and FPs towards haemoglobinopathy carrier screening on the basis of ethnicity.

Methodology: A structured questionnaire based on the Theory of Planned Behaviour sent to 1800 midwives and 2100 FPs

Results: 1346 questionnaires were collected (35% response). Midwives and FPs were positive about offering ethnicity based screening but do not currently carry this out. Factors explaining intention were social norm, control over the ability to carry out the test and social norm (R^2 0.522). The main factor explaining intention was social norm: their perception of negative peer opinion. If screening would become national policy most midwives and FPs would carry out ethnicity based haemoglobinopathy carrier screening. Both practitioners favoured ethnicity registration for health purposes.

Conclusion: Whether ethnicity based haemoglobinopathy screening is offered, is partially influenced by FPs and midwives perception of their colleagues' opinion about carrier testing based on ethnicity. However most are willing to offer screening should this become national policy. In developing new policy, debate amongst midwives and FPs should be encouraged articulating the voices of colleagues who already actively offer haemoglobinopathy screening.

P02 Clinical genetics and Dysmorphology

P02.001
Genomic and clinical characteristics of six patients with partially overlapping interstitial deletions at 10p12p11

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With the clinical implementation of genomic microarrays, the detection of cryptic unbalanced rearrangements in patients with syndromic developmental delay has considerably improved. Here we report the molecular karyotyping and phenotypic description of six new unrelated patients with partially overlapping microdeletions at 10p12.31p11.21 ranging from 1.0 Mb to 10.6 Mb. The smallest region of overlap is 306 kb, which includes *WAC* gene, known to be associated with microtubule function and to play a role in cell division. Another patient has previously been described with a 10 Mb deletion, partially overlapping with our six patients[1]. All seven patients have developmental delay and a majority of the patients have abnormal behaviour and dysmorphic features including bulbous nasal tip, deep set eyes, synophrys/thick eyebrows, and full cheeks, while other features varied. All patients also displayed various visual impairments and 6 out of 7 patients had cardiac malformations. Together with the previously reported patient, our study suggests that the detected deletions may represent a new contiguous gene syndrome caused by dosage sensitive genes that predispose to developmental delay.

1)Shahdadpuri R, de Vries B, Pfundt R, de Leeuw N, Reardon W: Pseudoarthrosis of the clavicle and copper beaten skull associated with chromosome 10p11.21p12.1 microdeletion. *Am J Med Genet A* 2008; 146A: 233-237.

P02.002
Vermis hypoplasia as part of a contiguous gene syndrome in a girl with microdeletion 10q26

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Microdeletions of 10q26 cause a contiguous gene syndrome described in about 60 patients until now. There is significant variation in deletion size and phenotype. Clinical symptoms include mental retardation, motor and speech delay, strabismus, characteristic facies, microcephaly, short stature, as well as in some cases feeding difficulties, behavioural problems, muscular hypotonia, ataxia, hearing loss, genitourinary anomalies and heart defects. We report on a 10-year-old girl who presented with moderate global developmental delay, severe speech delay, strabismus, intention tremor, ataxia, hydronephrosis

and mild dysmorphic features. Pregnancy was complicated by vaginal bleeding in gestational week 32. Furthermore, the mother recognized reduced fetal movements. Sectio was performed in gestational week 41 (APGAR score 3/8/9 at minutes 1/5/10, birth measurements within normal range). Developmental milestones were delayed with sitting at 8 months, walking at 22 months, first words at about 28 months. At the age of 30 months a brain MRI showed marked hypoplasia of the inferior part of the vermis cerebelli. Investigations regarding inborn errors of metabolism, congenital disorders of glycosylation as well as classical karyotyping were unremarkable. However, array CGH analysis revealed an interstitial 6.92 Mb microdeletion in 10q26. Further investigations showed that the deletion had occurred de novo. This is the first case of a 10q26 microdeletion with an abnormal MRI of the cerebellum. We hypothesize that the vermis hypoplasia might be causative for the ataxia and intention tremor in our patient.

P02.003**

Reciprocal extreme BMI phenotypes associated with gene dosage at the 16p11.2 locus

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Both underweight and obesity have been associated with increased morbidity and mortality. Weight faltering in childhood (or failure to thrive) have long-lasting deleterious effects on development, including adverse intellectual outcomes. In contrast to obesity, molecular abnormalities underlying low-weight remain elusive. While hemizygosity of a 600 kb region on the short arm of chromosome 16 (chr16:29.5-30.1Mb) causes a highly-penetrant form of obesity often associated with hyperphagia and intellectual disabilities, the corresponding reciprocal duplication is associated with underweight. We identified 108 carriers of this 16p11.2 duplication among 95,000 individuals. These carriers show significantly reduced weight (mean Z-score -0.6; $p<10^{-3}$) and adults have an 8.7-fold ($p<10^{-10}$) increased risk of being clinically underweight (defined by a BMI<18.5kg/m^2). An early onset gender-specific effect was found in cases ascertained through developmental and intellectual disabilities, with half of the boys younger than 5 years exhibiting failure to thrive. These features are only detectable postnatally and are associated with an unusually high frequency of selective and restrictive feeding behaviours. Duplication carriers also have a significant reduced head circumference ($p<10^{-5}$) which positively correlates with BMI. Weight, BMI and head circumference phenotypes as well as food intake behaviours are the converse of those reported in carriers of deletions at this locus, correlating with changes in transcript levels for genes mapping within the CNV, but not within flanking regions. The reciprocal impact of these 16p11.2 copy number variants evidences that severe obesity and being underweight can have mirror etiologies, possibly through contrasting effects on eating behaviour.

P02.004

Auxological evaluation in patients with a 22q11.2 microdeletion syndrome: normal prevalence of obesity but neonatal length and gender influence on body mass index evolution

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Aims: to evaluate auxological parameters in children and adults with a 22q11.2 microdeletion syndrome (22q11.2DS); to compare prevalence of obesity to that in the general French population.

Methods: 102 patients with 22q11.2DS (49 males, 53 females) were recruited from birth to adulthood through a reference center from Southern France.

Results: Mean Z score BMI and mean height were normal (0.07 ± 1.49 SD, -0.87±1.36 SDS, respectively). 16.1 % of patients were overweight (including obese), 57% of them were born SGA for length versus 25% of non overweight patients. During infancy, boy's BMI dramatically increased (+ 1.07 SD Z score) until normalization whereas girl's BMI increased from 2 to 4 years. Childhood: 14.7% were overweight, a prevalence similar to that in French children population. Adulhood: from 26 available measures, 19.2% were overweight. Male gender was significantly associated with a lower post pubertal BMI than female (β=-1,987). After 15 years of age, BMI and Z score of BMI were inversely correlated with neonatal length (β=-1.140) but positively associated with neonatal weight (β=+2.294) and female sex ($p<0.01$). From analysis of neonatal data, 22q11DS newborns were significantly shorter (-1.46± 1.2 SDS) with regard to their weight (-0.83 ±0.98 SDS) ($p<0.01$), even though mean neonatal measures were above -2SDS.

Conclusions: Our study did not find a higher prevalence of overweight in 22q11.2DS to that in French population. Post pubertal BMI was inversely correlated with neonatal length but positively associated with neonatal weight and female gender.

P02.005

3.7 Mb tandem microduplication in chromosome 5p13.1-p13.2 associated with developmental delay, macrocephaly, obesity, and lymphedema. Further characterization of the dup(5p13) syndrome.

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In a male patient with developmental delay, autistic behaviour, obesity, lymphedema, hypertension, macrocephaly, and facial features (dolichocephaly, short neck, low set posterior hairline, dysplastic ears, broad nasal root, short nose, microretrognathia) of chromosome 5p duplication (trisomy 5p) a 3.7 Mb de novo tandem microduplication of 5p13.1-13.2 (rs4703415-rs261752, i.e., chr5:35.62-39.36Mb) was identified. This observation contributes to the characterization and dissection of the 5p13 duplication syndrome. A comparison with previous case reports is provided. Similar to the patients with duplication of the entire NIPBL gene reported by Yan et al (2009) but different from several case reports on 5p13 duplication, there was, for instance, no heart defect in our patient. The possible role of genes within the duplicated region, especially of NIPBL, is discussed.

P02.006

Application of array CGH method in two 18q21.31-q23 deletion patients

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INTRODUCTION:

Partial deletions of the long arm of chromosome 18 lead to different clinical presentations. Patients whose deletions localized within the same region may exhibit distinct phenotypes. Conversely they can exhibit common clinical features. New molecular techniques such as array CGH allow for a more precise determination of deletion points leading to better-defined genotype-phenotype correlations. This method proved to be effective in determining molecular profiles with a resolution 5-10 times higher than conventional karyotyping methods.

MATERIAL- METHOD:

We had two patients admitted to our clinic; one of them, 13 years old boy with dysmorphic features and autism; the other 15 years old girl with inability to speak. We used array CGH method for diagnosis of these two patient. DNA samples which labelled according to the protocol, were hybridized with CytoSure Syndrome Plus(v2)4x44K microchips and scanned with Agilent Microarray Scanner. Obtained data were analyzed using Cytosure Analysis Software,v.2.0.8.

RESULTS:

According to array CGH analysis, 8.21 Mb deletion was detected in 18q21.31-q23 region. Important genes for this region are MALT1, RAX, LMAN1, MC4R, TNFRSF11A, BCL2, CTDP1 and FVT1. The conventional karyotype analysis was performed to these patients and 46XYdel(18)(q21.3-q23) was found. These results supported array CGH results.

CONCLUSION:
Although these patients possesed same deletions at the same region, their clinical manifestations was different and they had few common phenotypic features. Effects of the genes to the phenotype at this region is unknown but can be possible to investigate. These genes will provide new clues for pathogenesis and treatment of the disease.

P02.007
45,X/46,XY mosaicism: a cause of short stature in males

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45,X/46,XY mosaicism is associated with a broad spectrum of phenotypes ranging from apparently normal male development, individuals with incomplete sexual differentiation and males and females with clinical signs of Turner syndrome. The most common presentation for individuals with a 45,X/46,XY karyotype is sexual ambiguity, accounting for ~60%, and the least common category of 45,X/46,XY patients consists of those with bilaterally descended testes, found in 11-12%.
We report on two patients with an apparently normal male phenotype and 45,X/46,XY mosaicism who were diagnosed postnatally because of short stature.
Both of these boys presented at the age of 15 years with short stature, minor Turner-like stigmata, normal male external genitalia and spontaneous pubertal development. One of them had coarctaction of aorta with bicuspid aortic valve, an uncommon clinical feature in boys with 45,X/46,XY karyotype. The same patient had a trial of GH replacement therapy with poor response and his sperm analysis revealed azoospermia.
Like our patients, most mosaic 45,X/46,XY children with bilateral scrotal testes go unrecognised at birth and throughout childhood unless they have somatic features of Turner syndrome or significant growth retardation. We recommend that boys with otherwise unexplained short stature being short for their families should be karyotyped routinely as is recommended in short-stature girls. In addition, boys with 45,X/46,XY karyotype must be routinely followed-up for their potential to respond favourably to GH treatment and for late onset abnormalities, such as infertility and gonadal tumors.

P02.008
Cephalometric evaluation in a patient with 48,XXXX syndrome

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The aim of this study is to investigate the cephalo-facio-dental relationships in the craniofacial complex and the use them for diagnostic and orthodontics treatment. We studied the changes of the cephalo-facio-dental relationships, using serial lateral cephalometric roentgenograms in a 29 years old female with 48,XXXX karyotype. By using cephalometric methods we evaluated 32 linear and 12 angular measurements of the craniofacial complex. We found smaller linear and larger angular measurements. Even if all face heights were diminished, we recorded a significantly shortening of the length of the anterior and posterior cranial bases, posterior face height, the length of the calvarium and mandibular ramus length, while the mandibular corpus demonstrated only a slight reduction. This can explain the significantly increased SNB angle as a compensatory mechanism between the accentuated shortening of the anterior and the slight reduction of mandibular corpus, ramus height reduction of was compensated by a smaller gonial angle. The angles between the palatal and occlusal planes and between the palatal and mandibular planes were increased due to the fact that the palatal plane that was canted upwards. The patient was included in skeletal Class III growth pattern and associated a modified dentoalveolar complex with upper incisors having a reduction of the proclination and lower incisors in retrocline position. We concluded that even if the underlying mechanisms are not known, the presence of supernumerary X chromosome influence the development of craniofacial complex conducting to smaller linear parameters and enlarged angular measurements.

P02.009
Phenotypic variations in Wolf-Hirshorn syndrome

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Wolf-Hirshorn syndrome is rare chromosomal disorder caused by terminal deletion of the short arm of chromosome 4. The haploinsuficiency of the deleted region is expressed as severe phenotype which includes growth retardation, severe mental retardation, characteristic face, seizures and midline defects in the brain, heart, palate, hypospadias, etc. The severity of clinical presentation is due to the size of the deleted region, as well as genes involved in the deleted region.
We present five children with Wolf-Hirshorn syndrome with variable clinical presentation. Present dysmorphic stigmata as Greek helmet-like forehead, hyperthelorism, broad nose, short philtrum, low-set dysplastic ears, and sacral dimple were classified as minor, mild and severe. Aditional congenital anomalies of the heart, kidneys, genitalia and intestine were taken into the consideration. Seizures were present in 4 children, and were of variable type. Two of the children have visible cytogenetic deletion, two had microdeletion detected with FISH probe, and one child with less characteristic clinical picture had mosaic type of the deletion.
The phenotype of the deletion of the short arm of cheomosome 4 is variable -from minor to full blown clinical presentation with characteristic facial dysmorphism and adjacent malformations. Characteristic appearance resembling “Greek helmet” facies is the basis for establishing the diagnosis of Wolf-Hirschhorn (WH) syndrome. Since it is a contiguous gene syndrome, different regions of chromosome 4p could be deleted. Recently used molecular techniques increase the number of diagnosed cases due to the detection of smaller deletions.

P02.010
4p15 deletion in a patient with apparently isolated metopic craniosynostosis.

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Craniosynostosis, premature fusion of one ore more cranial sutures, affects approximately one in 3000 live births. Syndromic trigonocephaly has been associated with chromosomal imbalances or point mutations in single genes. Recently, it has been suggested that rare copy number variants (CNVs) in patients with isolated single-suture synostosis could contain genes important for cranial development. In fact, some of the involved genes play a role in osteoblasts proliferation, apoptosis and differentiation, whose deregulation can evolve in precocious suture closure. We report the case of a 12 months old boy with an apparently isolated craniosynostosis and harboring a de novo microdeletion in the cytoband 4p15. The imbalance, about 4 Mb in size is, up to date, the smallest deletion described in this region, encompassing 12 genes. SLIT2, originally characterized in the nervous system as a guidance factor for axons, looks interesting because it was recently described to have a role in in vitro osteoblast growth regulation. Further cases of CNVs including SLIT2 would be extremely useful in determining whether its deregulation could be implicated in premature suture closure. Among others, DCAF16 and NCAPG should also be considered for genetype phenotype correlation. In fact, these genes are involved in fundamental cell biological processes, and could be eventually associated with poor brain development. Up to now, developmental steps of our patient looked adequate for his age, but further follow up is needed in order to assess his psychomotor development. Genotype-phenotype comparison with other previously described cases with 4p15 deletion will be discussed in detail.

P02.011
Early-onset polycystic kidney disease (PKD) - a crucial additional finding of 4q deletion syndrome

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Several reports describing chromosome 4q deletion have been published since 1980’s . In 2010, 9 cases with 4q deletion were

reported by Bonnet et al and the name of 4q deletion syndrome was established. This is a contiguous gene deletion syndrome showing several characteristic features including severe growth retardation, teeth defect and neurosensorial deafness. *PKD2* gene which was mapped at 4q21-q23 by Mochizuki in 1996 is related to autosomal dominant type PKD. *PKD2* gene is deleted in 4q deletion syndrome. However, few reports have described renal cysts in this syndrome. We assumed that deleted *PKD2* should result in autosomal dominant PKD.

Here we report on 2 cases of 4q deletion syndrome; one is a 15-year-old female patient who was previously reported by Harada in 2002 and the other is a 9-year-old female patient showing deletion 4q21-21.3 and 4q21.1-22.2, respectively. Both of them showed several typical characteristic findings of 4q deletion syndrome including severe growth and mental retardation, and teeth defect. In addition to these features, both of them showed severe PKD by echogram and MRI without renal dysfunction. Usually *PKD2* mutation develops PKD frequently in adulthood and rarely in childhood. Our cases with this deletion developed PKD in childhood, suggesting that early onset may be related to the deletion, not a mutation, of *PKD2*.

PKD should be added to the essential features of 4q deletion syndrome. Clinicians should perform renal imaging study in patients with this syndrome even without abnormal findings in routine blood tests and urinalysis.

P02.012

Dysmorphic features, short stature and deafness explained by a homozygous deletion of 15p15.33 involving the SLC12A7 (Kcc4) gene and the NKD2 gene.

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A fourth child of consanguineous parents was born with a Pierre Robin sequence and dysmorphic features concerning round eyes, low set- and cup shaped ears, flat midface, bowed tip of the nose, underdeveloped nostrils and flat philtrum. Growth on - 2 SD, no heart or kidney anomalies. Normal karyotype, no 22q11 deletion.

The girl developed short stature and perceptive deafness. DFNB1, SHOX and CHARGE were excluded. A skeletal survey was normal.

Array CGH (Agilent 180k) showed a homozygous deletion of 50 kb of 5p15.33 involving the 3' end of both the NKD2 and SCL12A7 (Kcc4) gene. The healthy parents and three sibs are heterozygous carriers of the deletion.

Kcc4 is a K^+- Cl^- cotransporter playing a role in the K^+ transport in the inner ear and in the Cl^- transport in the proximal tubules of the kidney. Mice lacking Kcc4 are deaf, have a low bodyweight and develop renal tubular acidosis.

Nkd 1 and *nkd2* inhibit wnt-signaling and are involved in morphogenic signaling. In mice *ndk2* is expressed in the snout and ears. In the *ndk1-ndk2* knockout mouse the nose is short.

In conclusion, this patient has a homozygous deletion syndrome in which the loss of *Kcc4*, likely explains her deafness and short stature, and the loss of *nkd2* causes specific dysmorphic facial features.

P02.013

De novo 9p duplication in a syndromic child

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Background. 9p duplication syndrome is a rare chromosomal disorder often with well characterized clinical features. Array Comparative Genomic Hybridization (aCGH) have revealed that duplicated areas on 9p may have different clinical impact, i.e. 9p24.3 to 9p23 as a potential autism spectrum disorder locus, 9p22.3 to 9p22.2 as the critical region for the 9p duplication syndrome, 9p22.1 to 9p13.1 as a probable normal variant and 9p11.2 to 13.1 definitely as a normal variant

Methods. We describe a child with *de novo* 9p duplication and the genetic analyses performed in the family.

Results. Both chromosomal analysis and array Comparative Genomic Hybridization (aCGH) identified the duplication.

Conclusions. ACGH makes it easier to assess different clinical aspects such as developmental delay, autism and deformities with regard to the chromosomal findings.

P02.014***

Evidence for AXIN2/IRF6 interaction in oral facial clefting

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Oral-facial clefts (OFC) have a multigenic etiology that involves many different signaling pathways. We recently reported that families segregating OFCs present an increased history of cancer when compared to control families, and that AXIN2 is associated with OFCs in a US population. Since Axin2 is an important negative regulator of the Wnt pathway, and the Wnt pathway is integral to craniofacial development, we expanded our initial study to explore the association of AXIN2 markers with OFCs in 724 cleft families from North America, Europe, Asia, and Latin America. Thirteen AXIN2 SNPs were genotyped using TaqMan chemistry and tested for association with OFC using the Family-Based Association Test (FBAT). An association between SNP rs7224837 and OFC was identified in the pooled populations (P=0.001), while SNP rs3923086 showed association with Chinese families from Beijing and Shanghai presenting cleft lip and palate individuals only (P=0.004). We found statistical evidence of interaction between these AXIN2 SNPs and IRF6 SNPs rs2235371 and rs642961 (P<0.05). We assessed mouse Axin2 expression during mouse palatogenesis, and also detected co-localization of Axin2 and IRF6 proteins in the palate, oral and molar tooth epithelia. Taken together, our results support an important role for AXIN2 during craniofacial and palatal development. Further, these results provide insights into potentially etiologic AXIN2-IRF6 interactions that may contribute to the etiology of OFCs in humans.

P02.015**

COL4A2 mutation associated with familial porencephaly, aneurysm and secondary developmental anomalies

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Background. Familial porencephaly results from cerebral stroke during pregnancy or around birth and has been described with dominant mutations in the gene encoding for type IV collagen alpha 1 (*COL4A1)*. We observed three porencephaly families that lack *COL4A1* mutations. Mice harbouring mutations in either *Col4a1* or *Col4a2* suffer from cerebral and ocular bleeding and developmental defects. We hypothesized that *COL4A2* mutations could cause porencephaly in families without *COL4A1* mutations.

Methods. We sequenced *COL4A2* in three families with porencephaly, and characterized clinical, neuroradiological and cellular phenotypes by biochemistry and EM of skin.

Results. Sequencing of *COL4A2* identified the heterozygous pathogenic mutation Gly1389Arg and two probable pathogenic c.3206delC and Met813Leu in three families with cerebrovascular disease. In patients with mutations, phenotypes varied including porencephaly, microcephaly, white matter tract abnormalities, secondary cerebellar and optic nerve hypoplasia, stroke at adult age and carotid aneurysm. However, in two families, patients without the mutation were described. In support of pathogenicity, fragmentation and duplication of epidermal basement membranes was observed by electron microscopy in c.3206delC and Met813Leu patient skin biopsies, consistent with abnormal collagen IV network. Cultured fibroblasts of one c.3206delC patient showed significantly increased rates of apoptosis under stress conditions, but, interestingly, no associated signs of ER stress.

Conclusions. Dominant *COL4A2* mutations are a novel risk factor for porencephaly, stroke and brain developmental defects. Basement membrane defects appear to underly pathology, but abnormal regulation of apoptosis may be a contributing factor.

P02.016
G1138A mutation in FGFR3 gene in patients with achondroplasia

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Introduction. Achondroplasia is a common form of chondrodyplasia with a frequency of 1/15.000 - 40.000 new borns. It is transmitted by autosomal dominant trait with full penetrance but approximately 75-80% of the cases are caused by de novo mutations. The disease is determined by mutations in receptor-3 gene of the fibroblast growth factor (FGFR3), mapped to band 4p16.3, resulting in decreased endochondral ossficication, inhibited proliferation of chondrocytes in growth plate cartilage and decreased cartilage matrix. The most frequent mutations (up to 99%) are G1138A and G1183C. The aim of this study is to establish the prevalence of G1138A mutation in FGFR3 gene in patients with achondroplasia in our care.

Patients and method. The study group consisted of 27 patients (17 girls and 10 boys), aged between 1 year 8 months - 22 years, who were registered in the Centre of Genetic Diseases of First Pediatric Clinic Cluj in the period 2007-2010. The method consisted in: clinical assessment and radiological examinations (radiograms of the skull, upper and lower limbs, spinal column and pelvis). The DNA analasys was performed by PCR-RFLP technique.

Results. Between 2007 - 2010, 80 patients were diagnosed with bone dysplasia; 27 of them (33,75%) were diagnosed (on clinical and radiological basis) with achondroplasia. Out of this group, 13 patients (48,14%) were identified as heterozygotes for G1138A mutation in FGFR3 gene; this prevalence is very low comparatively with other studies (Hung CC, Alderborn A).

Conclusions. It is the first study that reports the prevelance of this mutation in Romania.

P02.017
Acro-Cardio-Facial syndrome: description of a new case.

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Acro-Cardio-Facial syndrome (ACFS) is a very rare genetic condition with nine patients been published until now. ACFS is clinically heterogeneous, but split-hand and split-foot malformation and congenital heart defect (CHD) are the most important diagnostic features. Other malformations and clinical signs reported are: cleft lip/palate, genital anomalies, facial dysmorphisms and mental retardation. Genetic cause is unknown and autosomal recessive inheritance is supposed.

We describe a male born to non-consanguineous parents, with hand malformations, CHD and peculiar morphologic facial features. Fetal ultrasound at 20 week gestation showed a complex CHD. At birth, he presented with cleft left hand and lack of flexion at the interphalangeal joints of the fifth right hand finger. On echocardiography the CHD detected consisted of truncus arteriosus type 1, ventricular septal defect and pulmonary atresia. Morphologic features of the face were: high forehead, broad nasal bridge, long philtrum, microretrognatia and large and dysmorphic low-set ears. TC scan showed the presence of schisis of the posterior arch of the lumbar vertebrae. Karyotype was 46,XY, and array-CGH is ongoing.

We underline the clinical variability of this condition and the wide range of malformations reported requiring a multidisciplinary approach. Clinical genetic assessment is important in order to define the diagnosis and genetic counselling is recommended for parents for high recurrence risk.

P02.018
Apert Syndrome With Fused Thalami

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An increased nuchal translucency had been detected at 12 weeks of gestation and second level ultrasounds (2-D, 3-D and 4-D) at 20+1 weeks of gestation revealed multiple cranio-facial dysmorphisms including turricephaly, frontal bossing, midface hypoplasia and ocular hyperthelorism. The pregnancy was terminated at 20+3 weeks of gestation.

External examination of the female fetus revealed a clinical phenotype indicative of Acrocephalosyndactyly type I (Apert Syndrome), confirmed by molecular analysis of the FGFR2 gene.

In addition, due to a transvaginal ultrasound detection of probable holoprosencephaly, a post mortem MRI scan was preformed revealing a minimal form of holoprosencephaly with fusion of the thalami.

Apert syndrome and holoprosencephaly occur with prevalences of approximately 1:55000 and 1:8000, respectively. Various central nervous system anomalies, such as ventriculomegaly, corpus callosum anomalies, hydrocephaly, defective septum pellucidum and mesial temporal abnormalities have been reported in patients with Apert syndrome.

While there are numerous patients affected simultaneously by HPE and craniosynostosis (one of the cardinal signs of Apert syndrome), cases of holoprosencephaly in Apert syndrome have never been reported so far.

P02.019
Subtle risk of dissection in thoracic aneurysms associated with mutations of smooth muscle alpha-actin 2 (ACTA2)

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To evaluate the prevalence and phenotype of smooth muscle alpha-actin (ACTA2) mutations in nonsyndromic thoracic aortic aneurysms and dissections (TAAD).Observational study of ACTA2 mutations in TAAD.Setting : Centre for Inherited Cardiovascular Diseases.

Patients A consecutive series of 100 patients with TAAD. Exclusion criteria included genetically confirmed Marfan syndrome, LoeyseDietz type 2, familial bicuspid aortic valve and EhlerseDanlos type IV syndromes.

Multidisciplinary clinical and imaging evaluation, genetic counselling and testing of ACTA2, and family screening.

Main outcome measures Prevalence of ACTA2 mutations and corresponding phenotypes.

Results TAAD was familial in 43 cases and sporadic in 57 cases. Five mutations in the familial TAAD group (12%) were identified that were absent in controls. The known p.Arg149Cys and the novel p.Asp82Glu, p.Glu243Lys and p.Val45Leu mutations affected evolutionarily conserved residues. The IVS4+1G>A mutation was novel. Of 14 affected relatives, 13 were carriers of the mutation identified in the corresponding proband while one deceased relative had no genetic test. Type A dissection was the first manifestation of aortic aneurysm in four probands and occurred unexpectedly in five relatives. The aortic aneurysm was age dependent and absent in mutated children. Of nine patients who had acute dissection, five died following surgery. At dissection, the size of the aortic aneurysm ranged from 40 mm to 95 mm. Extravascular, ocular, skeletal, nervous and pulmonary traits were variably associated with TAAD, with iris flocculi being most common.
Conclusions Timely diagnosis of TAAD in the probands, genetic counselling and family screening identify predisposed relatives and prevent catastrophic aortic dissections.

P02.020
Four new cases of Acute Necrotizing Encephalopathy caused by mutation in RANBP2 gene

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Acute necrotizing encephalopathy (ANE), first described in East Asia, affects mainly young and healthy children who develop acute encephalopathy, seizures and rapid progression to coma 2 or 3 days after the onset of a viral illness. An autosomal dominant form, ANE1, has been recognized and linked to mutations in the gene encoding the nuclear pore protein Ran Binding Protein 2 (RANBP2). We report the clinical, MRI, and molecular genetic findings of ANE1 in 4 non-related French patients. In all patients, the disease was manifested as a rapidly progressing acute febrile encephalopathy. The patients were previously neurologically normal, with the exception of one child who had residual neurologic problems following a previous febrile encephalopathy. Interestingly two of them were initially referred with the diagnosis of mitochondrial disease because of oxidative phosphorylation deficiency demonstrated in muscle biopsy. The disorder was fatal in two patients. The two other patients recovered from the coma but experienced significant neurologic disability. Cerebrospinal fluid protein was consistently elevated among patients during the acute illness. Imaging findings showed multiple symmetrical lesions affecting primarily the brainstem, the thalami and the external capsule. The four patients were heterozygous for the same missense mutation (c.1880C/T, p.Thr585Met) in the gene (RANBP2). The mutation occurred de novo in two patients and was inherited from healthy parents in two. These observations emphasize the distinctive brain MRI, the possible role in energy maintenance and the not fully penetrant phenotype associated with RANBP2 mutations.

P02.021
Consolidated clinical genetic testing for Aicardi-Goutières syndrome and related conditions: a service package covering *TREX1*, *RNASEH2B*, *RNASEH2C*, *RNASEH2A*, and *SAMHD1*.

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Clinical and molecular research in Aicardi-Goutières syndrome (AGS), a severe genetic encephalopathy resembling congenital infection, uncovered a range of immune-mediated brain and lupus-like conditions associated with dysregulation of nucleic acid metabolism and/or innate immunity mechanisms. Apart from classical AGS, the AGS spectrum comprises atypical AGS, retinal vasculopathy with cerebral leukodystrophy, chilblain lupus, and certain types of systemic lupus erythematosous, linked by a common genetic etiology. All can be caused by mutations in one, typically, of five known genes involved in nucleic acid clearance and/or innate immunity: TREX1, specifying the major 3'→5' DNA exonuclease in mammals; RNASEH2A, RNASEH2B, and RNASEH2C, encoding the components of the RNASEH2 RNA:DNA endoribonuclease complex; and SAMHD1. At least another, but minor, AGS-related gene is likely to await identification. The AGS system is of both biological and medical interest. For clinical use, we have developed and launched a comprehensive testing package consisting of high-throughput conventional sequencing of the coding regions of the above five genes. Our GeneTests-listed AGS service is estimated to have a clinical sensitivity around 80% and is available with a target turnaround time of 8 weeks for routine diagnostic requests; responding to urgent diagnostic queries is also possible. Overall, this cost-effective strategy reduces testing bias and enables more conclusive reporting while fully complementing, or even superseding, radiological, immunological, and biochemical investigations. Additionally, carrier and prenatal testing, in families with previously characterised mutations, is streamlined.

P02.022
A case of right-sided cardiac developmental abnormality due to a novel mutation in JAG1

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Introduction
The JAG1 gene encodes jagged-1 protein, a highly conserved ligand for the Notch1 receptor. Jagged/Notch interactions are critical for determination of cell fates in early development. JAG1 mutations cause the well-known Alagille syndrome (ALGS, MIM: #118450), but growing evidence implicates this gene in various non-syndromic right-sided cardiac defects (1).
Clinical case
We report on a case of a 4-year-old boy referred for evaluation of peripheral pulmonary stenosis. His weight and height were 20kg (0 SD) and 111cm (-1SD) respectively. Developmental milestones were normal. He showed some dysmorphic features: broad and prominent nasal bridge with bulbous nasal tip, long upslanting palpebral fissures and broad chin. There were no other clinical or radiological features of ALGS. Bidirectionnal sequencing of JAG1 (NM_000214.2) revealed a c.820G>T transversion in the exon 6, responsible for a p.Gly274Cys change in the second EGF-like repeat.
Discussion
The interest of this case is the link with a previously described large kindred with a JAG1 mutation in the same codon p.Gly274Asp (2). Interestingly, the affected family members presented striking similar facial features and right-sided cardiac defects, mainly Tetralogy of Fallot and peripheral pulmonary stenosis, but no other signs of ALGS. These findings suggest a genotype-phenotype correlation. Patients with right-sided cardiac defects should be carefully screened for dysmorphic features and family history of cardiac defects and even in the absence of other signs of ALGS, JAG1 should be screened.
References:
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P02.023
A case of misdiagnosis of Angelman syndrome, the role of MLPA genetic analysis in diagnosis

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Angelman syndrome (AS) is a neurodevelopmental disorder characterized by severe developmental delay or mental retardation, ataxia and dysmorphic facial features. The unique clinical features of AS manifest after 1 year of age, and it can take several years before the correct clinical diagnosis becomes obvious.
Microdeletions on chromosome 15q11.2q13 of maternal origin are the commonest genetic mechanism, occurring in approximately 70-75% of AS. Other mechanisms include paternal uniparental disomy, imprinting defects and point mutations or small deletions within the *UBE3A* gene. We report an interesting case of an 8-year-old male with MR, partial seizure, nystagmus and albinism. The EEG and MRI were abnormal. He showed a normal karyotype. The phenotypic presentation is not of

classical AS syndrome, however FISH analysis of PWS/AS had been performed in another laboratory, which showed no deletion.
Analysis of a DNA sample using the MLPA kit P297-B1 for detection of any interstitial microdeletion found no deletion. Subtelomeric microdeletion analysis using P70 and P36 kits showed loss of heterozygosity in the '15q11 probe' which corresponds to the AS/PWS region.
As these regions are in the critical region for PWS/AS, we further analyzed the region for imprinting defects and found an LOH of maternal origin. Further genetic and epigenetic analysis of the AS/PWS region was performed and confirmed an imprinting defect and LOH of the maternal allele in this patient. This genetic analysis confirmed the diagnosis of Angelman syndrome.
This is an interesting example of rigorous scientific work which could reveal a laboratory misdiagnosis and confirm the clinical diagnosis.

P02.024
Associated malformations among infants with anophthalmia and microphthalmia

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Infants with anophthalmia and microphthalmia very often have other associated congenital anomalies. The reported frequency and types of associated malformations vary between different studies.The purpose of this investigation was to assess the frequency and types of associated malformations among infants with an/microphthalmia in a geographically well defined population from 1979 to 2004 of 346,831 consecutive births. Of the 87 infants with an/microphthalmia born during this period (prevalence at birth of 2.5 per 10,000), 89.6 % had associated malformations. Infants with associated malformation were divided into recognizable conditions (22 (25.3%) infants with chromosomal and 15 (17.2%) with non chromosomal conditions), and non recognizable conditions (41 (47.1%) infants with multiple malformations). Trisomies 13 and 18 were the most frequent chromosomal abnormalities. Amniotic bands sequence, CHARGE association, Meckel-Gruber syndrome and VACTERL association were most often present in recognizable non chromosomal conditions. Malformations in the musculoskeletal, cardiovascular and central nervous systems were the most common other anomalies in infants with multiple malformations and non recognizable conditions. A de novo heterozygous loss-of-function point mutation in SOX2 was reported in 10 to 20 % of severe bilateral an/microphthalmia. The frequency of associated malformations in infants with anophthalmia and microphthalmia emphasizes the need for a thorough investigation of these infants. Routine screening for other malformations especially musculoskeletal, cardiac and central nervous systems anomalies may need to be considered in infants with anophthalmia and microphthalmia, and referral of these infants for genetics evaluation and counseling seems warranted.

P02.025
Analysis of SOX2, OTX2, and PAX6 genes in 89 Brazilian patients with severe eye malformation

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Anophthalmia/ microphthalmia (A/M) are major structural eye malformations. The best estimates of the birth prevalence of microphthalmia and anophthalmia from well maintained population based registers are 14 and 3 per 100 000 births, respectively. A/M is a heterogenous condition with various etiologies. One-third of individuals with A/M have associated malformations. Heritable causes of A/M include chromosome abnormalities and syndromic or nonsyndromic single gene disorders fashion. A/M is clinically and etiologically heterogeneous, and it has been estimated that only 20-40% of patients receive accurate molecular information regarding the pathogenesis of their malformation.
Homozygous mutations in the *PAX6* gene in the RAX and VSX2 (CHX10) genes have been described as causing A/M. Mutations in *SOX2* that segregate in an autosomal dominant pattern have been described in individuals with A/M. Mutations in OTX2 have been reported in up to 5% of individuals with A/M. Here, we analyzed *SOX2*, *OTX2*, and *PAX6* genes in 89 Brazilian patients with severe eye malformations who were ascertained from the Hospital for Rehabilitation of Craniofacial Anomalies, Bauru-SP, Brazil . They were divided in 9 groups: A/M + cleft lip and/or atypical cleft; Oculo-Ariculo-Vetebral Spectrum (OAVS); A/M isolated; Cerebro-Oculo-Nasal; Frontonasal dysplasia and/or Oculo-Ariculo-Frontonasal; Holoprosencephaly; Anoftalmia-Waardenburg syndrome; CHARGE.
No disease-causing mutations were identified in any groups. This study suggests that mutations in *SOX2*, *OTX2*, and *PAX6* do not appear to be a common cause of ocular defects other than anophthalmia/ microphthalmia. Further studies with larger numbers of different phenotypes may reveal mutations in these genes.

P02.026
Isolated congenital anosmia

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Which sense would you rather lose? A majority would answer smell. Despite this, smell has a major impact on our lives, i.e. related to food consumption, daily hygiene, detection of dangerous gasses and partner selection. Approximately 5% of the general population are anosmic, but although many are unaware of their condition, anosmia has repeatedly been related to loss or gain of weight, depression and generally a poor quality of life. The majority of individuals suffering from anosmia have an acquired condition, which develops throughout life due to head trauma, allergies, sinonasal disease etc. A much smaller minority (~1% of the anosmic population) have no recollection of ever being able to smell and are thus classified as having isolated congenital anosmia (ICA). A genetic origin is likely to explain the anosmia in this group, however human mutations have yet to be found. The scope of this review is to focus on the genetic basis of ICA through i) published families and cases with ICA, ii) anosmia in combination with other anomalies and iii) olfactory signal transduction pathway genes and animal models which may shed light on potential candidate genes and pathways involved. The lack of knowledge about specific genetic causes of ICA is a challenge but also an opportunity, which should now be within reach by application of the newest technological developments in genetic and genome research.

P02.027
One mutation fits all: Phospholamban R14del underlies both arrhythmogenic right ventricular cardiomyopathy and dilated cardiomyopathy

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Background Although considered separate entities, arrhythmogenic right ventricular cardiomyopathy (ARVC) and dilated cardiomyopathy (DCM) show considerable clinical overlap. We hypothesized that phospholamban gene (*PLN*) mutations could underlie both DCM and ARVC.
Methods We screened 240 DCM and 97 ARVC index-patients for *PLN* mutations and evaluated clinical characteristics. Immunohistochemistry (IHC) was performed in 16 myocardial samples from *PLN* mutation carriers.
Results Mutation R14del in *PLN* was identified in 31 of 240 (13%) DCM and 12 of 97 (12%) ARVC index-patients. This mutation was also present in an ARVC cohort from a different continent. Haplotype analysis revealed a common founder mutation, estimated to be 575-825 years old. A low voltage ECG was present in 42% of R14del+ patients. R14del+ DCM patients more often demonstrated: appropriate ICD discharge (42% vs. 9%, P<0.001), cardiac transplantation (19% vs. 3%, P<0.01), and a family history for sudden death (35% vs. 14%, P<0.01), when compared to R14del- DCM patients. In ARVC patients, these differences were not significant. IHC revealed absent/depressed levels for the desmosomal protein plakoglobin at intercalated disks in 5 of 7 (71%) R14del+ ARVC samples and in 1 of 9 (11%) R14del+ DCM

samples (P=0.03).
Conclusions The *PLN* R14del founder mutation underlies both ARVC and DCM in a substantial number of patients. R14del+ DCM patients demonstrate a severe phenotype. Reduced plakoglobin at the intercalated disk appears to track with the ARVC phenotype instead of the genotype. These findings challenge the distinction of DCM and ARVC as separate entities and support the concept of 'arrhythmogenic cardiomyopathy'.

P02.028
Molecular Genetic Investigation of the ATM gene in Iranian population

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Ataxia-telangiectasia (A-T) is characterized by progressive cerebellar ataxia beginning between ages one and four years, oculomotor apraxia, choreoathetosis, telangiectasias of the conjunctivae, immunodeficiency, frequent infections, and an increased risk for malignancy, particularly leukemia and lymphoma.
ATM is the only gene known to be associated with ataxia-telangiectasia. The normal gene has 66 (62 coding) exons and a 13-kb cDNA. More than 500 unique mutations are known. No common mutations ('hot spots') have been identified. Most affected individuals in Europe and USA inherit different mutations from each parent, i.e., they are compound heterozygotes. Most mutations result in absence of ATM protein.
In this study, peripheral blood samples from 5 patients were extracted by salt out method to isolate total genomic DNA. Methods were used for the detection of mutations were PCR amplification and direct sequencing.
We found in 5 Iranian AT patients a homozygous nucleotide deletion was identified in ATM gene. We are going to discuss about clinical and molecular aspects of our patients. We need further investigation to prove this mutation as founder effect in Iranian population.

P02.029
Antithrombin III defeciency; a case report

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Antithrombin III deficiency is first reported by Egeber et al. in 1965. The incidence for Japanese is 0.18% and is almost same as Caucasian. This lady is 30 y/o, G1P0 and has been followed up by vascular surgeon since she had deep vein thrombosis at 19 y/o. She is on low-dose aspirin since then and came to us because of her pregnancy.
When she came to us first time, she had doubted a bit of familial condition but not been sure about that. She mentioned her mother side uncle had cerebral infarction and her second cousin had lost her first child because of thrombosis. However, her mother does not have any history of thrombosis. We talked about the possibility of inheritance because of family history and gave her information about genetic test as well.
Her pregnancy went good and had a baby safely. The baby has been tested ATIII and ATIII activity which came back as normal. We talked about some worries of her mother with the patient and she wanted her mother to take a blood test for ATIII, but her mother declined. We finally did not have any chance to talk to her mother in this case.
It is still difficult to talk about familial condition sometimes in Japan. This could be big issue for us how to provide more information if there are some prevention of life threatening situation.

P02.030
Severe micrognathia, question mark ears - auriculo-condylar syndrome: case report

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Auriculo-condylar syndrome (ACS) is an autosomal dominant disorder of first and second branchial arches, with complete penetrance and great intra- and inter-familial phenotypic variation; ACS is characterized by dysplastic ears ('question mark ears'), micro-retrognathia, microstomia, mandibular condyle hypoplasia, prominent cheeks. The psyhological development is normal. The genetic defect for ACS is still unknown; the gene could be probably map in 1p21.1-q23.3 region.
We describe a pacient CPN, a 6 months old girl, with a highly suggestive dysmorphic face (severe micro-retrognathia, round facial appearance, prominent cheeks, dysplastic ears with abnormal lobule). The patient is the one child of a young and non-consanguineous couple. The mother revealed the same facial malformations as the child. We discussed a possible differential diagnosis.
We underline the importance for a good knowledge of the clinical features and syndromes of first and second pharyngeal arches for a correct diagnosis, clinical assessment and genetic counseling.

P02.031
New case of Primrose syndrome with mild intellectual disability.

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We report on a 27 years old man, who represents the sixth and the youngest published case of Primrose syndrome. Primrose syndrome (PS) (OMIM#295090) belongs to an extremely rare entities of unknown etiology characterized by the progressive wasting of distal muscles of the legs, the small muscles of the hands resulting in contractures, the presence of mental retardation, hearing problems, cataracts, brain calcification and the leading feature- ossification of ear cartilage. All the main manifestations, were present in our patient. Despite of strict phenotypic similarity to five other PS cases our patient represented with a mild mental retardation. Additionally we have found hypergonadotropic hypogonadism and a low bone density due to progressive osteoporosis. We discuss our observations in relation to previously published cases and we stress a need of the detail phenotypic descriptions of further cases as a syndrome remains a very rare finding and a genetic basis of PS is still undiscovered.

P02.032
ZEB2 missense mutations leading to an unusual presentation of Mowat-Wilson syndrome with moderate intellectual disability

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Mowat-Wilson syndrome (MWS, MIM#235730) is an intellectual disability-multiple congenital anomaly syndrome characterized by severe intellectual disability, epilepsy, agenesis of the corpus callosum, distinctive facial dysmorphism, heart defects, urogenital malformations and Hirschsprung disease. ZEB2 truncating mutations or deletions are disease causing. SIP1 (Smad Interacting Protein 1), the encoded protein, is a two-handed zinc finger/homeodomain transcriptional factor. Two separate clusters of zinc fingers have been characterized (in N-terminus and in C-terminus), and both must bind for transcriptional regulation. SIP1 target genes are numerous, and the best characterized is E-cadherin. SIP1 is early expressed by several tissues during embryonic development, including the neural crest, neuroepithelium and limb buds.
We report two patients with heterozygote missense mutations (c.3164A>G, p.Tyr1055Cys; c.3211T>C; p.Ser1071Pro) localized in the C-terminus zinc finger, and possibly resulting in ADN/protein interactions. We performed in vitro functional tests, using the luciferase reporter system and confirmed that both mutations abolished transcriptional repression of SIP1 on E-Cadherin. Both patients have a facial gestalt of MWS and milder intellectual disability and no congenital malformations.
In conclusion, we report an unusual clinical presentation of MWS with moderate intellectual disability and a distinctive facial appearance ascribed to ZEB2 missense mutations.

P02.033
Recurrent duplication Xp11.22-p11.23 can be associated with propensity to autoimmune disease: a case report and review of the literature

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Introduction. A recurrent 4.5 Mb duplication of Xp11.2 has been reported in females with variable degree of intellectual disability and language impairment associated with endocrine abnormalities, epilepsy and skeletal abnormalities.
Case report. The proband is the first daughter of healthy non-consanguineous parents. She was born at term with normal antropometric measurements. At age 10, she was diagnosed with a de novo 4,5 Mb Xp11.2p11.23 duplication syndrome because of mild intellectual disability and speech delay, hirsutism, obesity and epilepsy. At age 12, she presented with rapidly progressive proximal muscle weakness of arms and legs. A positive Tensilon test was suggestive for Myasthenia Gravis. EMG evaluation revealed low evoked compound motor action potentials, consistent with Lambert-Eaton myasthenic syndrome (LEMS). LEMS is a paraneoplastic disorder of neuromuscular transmission, based on auto-immunity to the voltage-gated calcium channel. Only rare cases have been reported in childhood. Reviewing the histories of other female patients reported with a similar Xp11.2 duplication revealed that 3 out of 11 patients suffered from (potential) auto-immune disease such as rectocolitis, Wegener's granulomatosis and hypothyroidism with hyperinsulinism.
Conclusion. The observation of a rare autoimmune myasthenic syndrome in a young patient with a Xp11.2 duplication in conjunction with previously reported autoimmune disorders suggest that carrier females of this recurrent duplication might have a propensity towards autoimmune disease. Some genes within the duplication are involved in either immune response (e.g. FOXP3) or calcium homeostasis (e.g. RGN) and could have a potential role in the occurence of auto-immunity in general or LEMS specifically.

P02.034
A 273-kb duplication at 22q13.33 encompassing the SHANK3 gene in 2 sibs with microcephaly, behavioral disorder and learning disabilities

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The 22q13 deletion syndrome is a not uncommon condition associated with global developmental delay, absent or delayed speech and hypotonia. Pure distal trisomy of the long arm of chromosome 22 are rare. Twenty patients, including our two cases, with variable clinical phenotype extending from mild psychomotor delay to severe delay with congenital malformations, have been shown to have a pure distal 22q trisomy. The size of the duplicated segment is extremely variable. Here we report on a brother and a sister presenting the smallest cryptic 22q13.33 duplication ever reported, detected by a salsa MLPA P188 kit 22q13. The duplicated region spans about 273-kb which encompasses 11 genes, including SHANK3. Both patients live in an institution because of the desertion of the parents and show developmental delay, especially in the language sphere, mild intellectual disabilities, behavioral disturbance, microcephaly, growth retardation and mild dysmorphic features. This phenotype was previously described in patients with larger duplication 22q13.3 and a recognizable phenotype was suggested. We confirm here this phenotype and delineate a critical chromosomal region of 273-kb, including the SHANK3 gene, which appears to be a strong candidate gene for the 22q13.33 duplication phenotype.

P02.035
De novo 7q deletion in a family with 16p duplication, autism and dyslexia

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Background. Copy number variations (CNV) in several chromosomal regions have been associated with increased risk of autism spectrum disorders (ASD). Chromosomal regions implicated in studies of ASD and associated phenotypes such as schizophrenia, mental retardation and developmental delay include 1p, 1q, 3p, 5q, 7q, 10q, 15q, 16p, 17p, 20p, 22q and Xq.
Methods. We describe a child with *de novo* 7q deletion and maternal 16p duplication and the genetic analyses performed in the family.
Results. The chromosomal analyses were normal while array Comparative Genomic Hybridization (aCGH) identified the 7q deletion and the 16p duplication.
Conclusions. ACGH makes it easier to assess different clinical aspects of neurodevelopmental disorders such as autism with regard to the chromosomal findings.

P02.036
Autism Spectrum Disorders and Seizure Syndrome

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INTRODUCTION: Autism and autism spectrum disorders (ASD) are serious early childhood neurodevelopment disorders with a genetic aetiology and increasing prevalence. Patients often have co-morbid disorders. 11-39% may have seizure syndrome. According to the literature, 42% of patients with autism, ASD and mental retardation have seizure syndrome. The aim of the study was to obtain information about patients with autism, ASD and seizure syndrome.
METHODS: 158 patients with autism and ASD were ascertained who had been treated in the Children's University Hospital, Child Psychiatry Clinic and Medical Genetics Clinic from 2006 - 2010. We recorded the patients' clinical symptoms (ICD-10), ADOS, electroencephalography (EEG) and IQ. SPSS 13 was used for statistical analysis. The frequency of seizure syndrome in both patient groups was calculated.
RESULTS: Seizure syndrome was identified in four patients with autism and 11 patients with ASD. Two patients in the autism group had their IQ within normal limits, four patients were moderately mentally retarded. In the ASD group, one patient had IQ within normal limits, four patients were mildly mentally retarded, four patients had severe mental retardation.
CONCLUSION: The incidence of seizure syndrome was 9.5% in both patient groups. Seizure syndrome is more frequent in patients with some degree of mental retardation than in patients with normal IQ.

P02.037
Sub-microscopic chromosomal imbalances in two children with idiopathic autism spectrum disorder

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Autism is a childhood neurodevelopmental disorder apparent by 3 years of age and characterized by qualitative impairments in reciprocal social interaction, deficits in verbal communication, restricted interests and repetitive behaviors. The exact aetiology of autism remains unknown, although it is likely to result from a complex combination of genetic, neurological, and environmental factors. Recent high resolution microarray-based studies in autism have identified a number of novel submicroscopic copy number variants (CNVs) including both deletions and duplications.
We report two children with autistic behavior and mental retardation. We have used genomic array CytoChip Oligo (BlueGnome, Cambridge, UK), format 2x105K, version 1.1. and BlueFuse Multi software, version 2.2. The 2x105K array detects 35 Kb imbalances on the backbone and has tiling of 20 probes over 137 OMIM disease loci. Array CGH-

analysis revealed cryptic deletion of 16p11.2 region spanning 513 kb in first patient and an amplification spanning 1,624 Mb of Xp22.31 region in second patient. Deletions and duplications at chromosome 16p11.2 appear to be associated with ~1% of unexplained, idiopathic and nonsyndromic autism. Several of the genes that reside within the 16p11.2 microdeletion represent promising candidates for autism based on known expression and functional data. Submicroscopic duplication of Xp22.31 has been reported as either a possible cause of intellectual disability and/or autistic behavior or a benign variant. . The Xp22.31 duplication included the steroid sulfatase (STS) gene.
Our results indicate that array-CGH is a powerful tool to detect sub-microscopic pathogenic imbalances in patients with idiopathic autism and/or mental retardation.

P02.038
Children autism and mitochondrial DNA mutations

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In the data of the WHO (World Health Organization) the prevalence of autism in children
is reported as 1-6 per 1000. The number in Bulgaria is currently unknown. But the hospitalization
of patients with speech, hearing and communication problems has increased in the past 10 years. Our experience in clinical genetics proved that childhood autism is a heterogeneous syndrome. The aim of our work was to analyze children with autism for mitochondrial DNA mutations and discuss the possibility of their treatment. We made clinical and laboratory investigations and sequenced mt DNA regions in peripheral blood by the PCR-SBT method in 4 of our patients with autism.
We found different mt DNA mutations and polymorphisms. In a girl suspected for Rett syndrome we found G6852A in mt-CO1,which changed Gly>Ser. In the 3 boys with autism we found the polymorphism T4216C (connected with predisposition to LHON or insulin resistance) in the first,
the polymorphism G9055A (protective of Parkinson disease) in the second, and a polymorphism connected with predisposition to LHON in the third. The children with mt DNA changes improved on treatment with a casein- and gluten-free diet and high dose vitamin Q10. We propose that children with autism should be investigated for mtDNA mutations and treated as mitochondrial disorders.

P02.039
Axenfeld-Rieger syndrome in a neonate with non-immune hydrops fetalis.

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Axenfeld-Rieger syndrome (ARS) comprises a spectrum of rare, heterogeneous autosomal dominant disorders mainly affecting the anterior segment of the eye. ARS leads to blindness from glaucoma in about 50% of patients. Extra-ocular clinical manifestations of ARS are highly variable and can include mid-face hypoplasia, dental anomalies and redundant umbilical skin. To date, several genes and loci on chromosomes 4, 6, 13 and 16 have been found to be associated with ARS, among them mutations in *PITX2* and *FOXC1*, which are responsible for the majority of ARS cases.
We are reporting a neonate who was born at 32 weeks due to non-immune hydrops and maternal mirror syndrome. Ocular findings in this patient consisted of bilateral anterior segment dysgenesis, glaucoma and posterior embryotoxon. Facial dysmorphic features included edema of head and face, depressed nasal bridge, hypertelorism, upturned nose and posteriorly rotated, low-set ears. MRI of the brain showed slightly small vermis, and echocardiogram revealed PFO and PDA.
No mutations were found on sequencing of *PITX2* and *FOXC1*, with MLPA studies pending. Microarray analysis revealed a duplication at chromosome 3p26 of unknown significance, and parental chromosomal studies are pending.
This case is the first report of an ARS patient with non-immune hydrops fetalis, further expanding the phenotypic spectrum in this group of disorders. To date, about 60% of ARS patients have no identifiable genetic defect. The highly variable phenotype of ARS necessitates further studies to identify the pathogenetic mechanisms that play a critical role in the development of the eye and other organs.

P02.040
Baraitser-Winter syndrome: A new Tunisian case

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Introduction: Baraitser-Winter syndrome is a rare multiple congenital anomalies characterized by iris coloboma, ptosis, hypertelorism, and mental retardation. It was first described by Baraitser and winter in 1988. The genetic mechanism underlying this syndrome has not been elucidated
Case report: We report the case of a 9-year-old male who was the third child of unrelated parents. He was referred to our genetic counseling service, since the age of 9 months, for evaluation of dysmorphic features and psychomotor delay
His face was characterized by brachycepahaly, hypertelorism, bilateral ptosis, short nose, long philtrum, micrognathia, malformed ears and short neck. Limbs examination showed valgus flat feets and genu varum. Evaluation of his mental development revealed a mild intellectual deficiency (IQ=62)
Ophtalmologic examination identified a right retino-choroidal coloboma and a bilateral optic nerve coloboma. Brain TDM demonstrated a bilateral atrophy of the frontal cortex. The remaining investigations including caryotype, cardiac and abdominal ultrasonographies were normal
Discussion: In this report we have presented a child with coloboma; ptosis; hypertelorism and mental retardation. This phenotype resembles the previous descriptions of patients with the Baraitser-Winter syndrome. Some of the peculiar craniofacial features (hypertelorism, bilateral ptosis,and short nose) which are highly characteristic and crucial to the diagnosis of the Baraitser-Winter syndrome are present in our patient. None of the multiple cerebral hemispheric malformations that have been described in the Baraitser-Winter syndrome like lissencephaly, pachygyria, cortical heterotopias or agenesis of the corpus callosum was described in our patient. This observation underlines the phenotypic heterogeneity of this syndrome.

P02.041
Mutation spectrum in Iranian patients with Bardet-Biedl syndrome

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Bardet-Biedl syndrome (BBS) is a rare genetic disorder that affects the brain and can cause multiple physical problems including a deterioration of the intellect and neurological functions. BBS is typically inherited in as autosomal recessive manner. Fourteen genes are known to be associated with BBS: *BBS1*, *BBS2*, *ARL6/BBS3*, *BBS4*, *BBS5*, *MKKS/BBS6*, *BBS7*, *TTC8/BBS8*, *B1/BBS9*, *BBS10*, *TRIM32/BBS11*, *BBS12*, *MKS1/BBS13*, and *CEP290/BBS14*. In some families, mutations in more than one BBS gene may result in a clinical phenotype of BBS. However, such families are difficult to identify and may account for less than 10% of all BBS patients.
The object of this study was to detect the mutation spectrum of BBS genes in our patients. probands of 10 Iranian families with clinical symptoms of BBS were tested for BBS1 and BBS2 gene mutations, which account for 23% and 20% of the mutations in BBS patients. Sequencing of *BBS1* and *BBS2* genes revealed a heterozygous mutation in one of the families, and 4 novel polymorphisms (c420, GCC>CCC, Ala>Pro, c424, AAA>AAT, Ala>Asn, c. 428 GCC>TCC, Ala>Ser & c.227 GAG>CAG, Glu>Gln) in *BBS1* gene. We could not detect any mutation except one polymorphism in *BBS2* gene.
Since we could not detect any mutations in the two most prevalent genes which account for over 40% of the mutations in BBS patients, our results suggest that other known or population specific genes may be involved in pathogenesis of BBS in Iranian population. Sequencing of the remaining genes is under way.

P02.042
Natural history, diagnosis and genetic aspects of Barth syndrome. New findings from a nationally commissioned service for Barth syndrome patients

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Barth syndrome (BTHS) is an x-linked disorder presenting as a triad of cardiomyopathy, neutropenia and 3 methylglutaconic aciduria.
A National clinic for patients and families with BTHS has been established in Bristol UK. This has allowed a comprehensive prospective clinical and genetic study of a cohort of boys with BTHS.
We have shown that BTHS is a multisystem disorder or syndrome. Facial dysmorphism gives BTHS boys a recognisable appearance. Other features include early short stature followed by later tall stature, a nonspecific skeletal myopathy and food fads. Dilated cardiomyopathy and left ventricular compaction both occur and conduction defects are common and a cause of sudden death. Transplantation in earlier life is successful. Neutropenia may be cyclical and responds to treatment with GCSF.
Pedigree analysis showed that families had a history of miscarriages, stillbirths and male neonatal deaths. One family had three pregnancies presenting as fetal hydrops in the mid trimester
Caused by mutations in TAZ , BTHS can be reliably diagnosed by measuring cardiolipin. This is a cheaper and quicker diagnostic test which can be followed by TAZ analysis in order to detect female carriers. Retrospective diagnosis in affected males has been made on samples from newborn screening tests.
Based on our figures for the prevalence of BTHS in the south west of England we suspect that BTHS remains and underdiagnosed condition and should be screened for in all boys presenting with cardiomyopathy and neutropenia and suspected in families with multiple unexplained male fetal loss or sudden death.

P02.043
Nevoid basal cell carcinoma syndrome: a report of a case

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Nevoid basal cell carcinoma syndrome (NBCCS), also known as Gorlin syndrome, is a rare autosomal dominant disease.It shows a high level of penetrance and variable expressiveness. This multisystem disorder characterized by multiple basal cell naevi, cysts of the jaw, pits of the palms and soles, skeletal anomalies, and various other defects. Patients with Gorlin syndrome have a predisposition to basal cell carcinomas and other neoplasms.
We present a patient with NBCCS who was diagnosed when he was 20 years old. He had a course face ,hoarse voice, hypertelorism, broad nasal bridge,pouting lips, parietal bossing with prominent supraorbital ridges and heavy and fused eyebrows . He was tall and marfanoid. On his physical examination, nevoid basal cell carcinomas were seen over his neck,shoulders,arms thrunk and face . He has developed basal cell carcinomas (BCCs) ,in particular on his lower eye lids and he was operated. He has palmar pits on his both hands. He was threated for dentical problems several times. He had malocclusion(i.e. displaced, impacted or missing teeth)and jaw cysts. He had pectus excavatum and synostic, bifid and partially missing ribs detected in his X-ray examination. Thoracolumbar spine films revealed scoliosis,too. Because of the autosomal dominant inheritance of this syndrome, the patient's family was then investigated. A multidisciplinary approach to management, together with periodic follow-up, are advocated for the general well-being of all NBCCS patients and their families.

P02.044
MS-MLPA technique in diagnosis of Beckwith-Wiedemann syndrome

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Beckwith-Wiedemann syndrome (BWS) is an overgrowth syndrome characterized by macroglossia, macrosomia, abdominal wall defects and predisposition to embryonal tumor development. BWS is caused by defective expression of imprinted genes (*IGF2*, *H19*, *CDKN1C*, *KCNQ1*, *KCNQ1OT1*) located on chromosome 11p15.5. The genes are functionally divided into two domains that are controlled by two Imprinting Centers: IC1 (H19DMR) and IC2 (KvDMR1). The most common defects in BWS patients are uniparental paternal disomy at 11p15.5 region, gain of methylation at IC1 and loss of methylation at IC2. Here we present six Polish patients with clinical diagnosis of BWS. Molecular analysis was performed by methylation sensitive multiplex ligation-dependent probe amplification using MS-MLPA BWS/RSS ME030-B2 kit (MRC-Holland). The kit enables determination of methylation status of the 11p15.5 region, as well as duplications or deletions in that region. The analysis demonstrated hypomethylation in KvDMR1 region in four patients, hypermethylation of H19DMR region in one patient and both KvDMR1 hypomethylation and H19DMR hypermethylation in one patient. Molecular analysis confirmed the diagnosis of BWS in all investigated patients. To our knowledge the presented study is a first attempt to introduce MS-MLPA in the molecular diagnosis of Polish BWS patients. We plan to conduct further studies to characterize the genetic background of BWS in Polish population.
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P02.045
A girl with the features of Binder syndrome and partial trisomy of the pericentromeric region of chromosome 5

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In 1962 Binder assigned maxillonasal dysplasia as a distinct entity characterized by midfacial hypoplasia, flat vertical nose, and malocclusion. The etiology of Binder syndrome (BS) is unknown. Here we describe a 10-year-old girl with facial dysmorphism characterized by features of BS, in whom a small ring from chromosome 5 was found. The proposita was born at term after uneventful pregnancy to non-consanguineous parents. She visited our genetic clinic at 10 years of age because of facial dysmorphism and moderate mental retardation. She had pointed chin, flat nasal bridge, left-deviated nasal septum, hypertelorism, mid-facial hypoplasia, blepharophimosis, prognatism, pectus excavatum and inverted nipples. Radiographic survey detected hypoplasic maxilla and ossis nasi, hypoplastic cervical vertebrae I-II. Psychological survey showed slight developmental delay. Chromosome analysis showed a small supernumerary marker chromosome, which showed clear ring form. FISH analysis confirmed that the marker chromosome derived from chromosome 5.
Array analysis revealed that the ring 5 chromosome contains a 19,168kb genomic region comprising cytogenetic bands 5p13.3 to p11.1., detailed analysis of the region lead to identification of 62 genes. Of these, three were disease associated dosage-sensitive genes, the nipped-B homolog gene (*NIPBL*), the oncostatin M receptor gene (*OSMR*) and the fibroblast growth factor 10 gene (*FGF10*). A causal relationship is suggested between these genes and BS.

P02.046
Microduplications 22q11.21 frequently associated with classic bladder exstrophy

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Classic exstrophy of the bladder (CBE) is part of the rare exstrophy-epispadias complex (BEEC), a spectrum of urogenital anomalies in which part or all of the distal urinary tract fails to close. Previously, four CBE cases with underlying microduplication 22q11.2 in a cohort of 102 BEEC patients have been reported (Lundin et al., 2010; Draaken et al., 2010).
Screening of additional 250 patients with CBE (223) as well as epispadias (9) and exstrophy of the cloaca (18) using multiplex ligation-dependent probe amplification (MLPA) analysis identified additional five CBE patients with a microduplication 22q11.2. Paternity was confirmed in all families. Among the nine cases the duplication had been inherited three times from an unaffected parent (two mothers, one father). All duplications differ in size ranging from about 0.07 Mb to 2.71 Mb. MLPA results were confirmed using Illumina's Beadchips thereby specifying the maximum region from SNPs rs2543958 to rs12484186. According to hg18 this region on chromosome 22 (nt:17,257,787-19,965,324) comprises 2.71 Mb, harbouring 47 genes as well as the sequence information of at least four microRNAs.
Given these observations, we were able to confirm the association of causally underlying microduplications 22q11.2 in cases of isolated CBE and hypothesize that a single phenocritical gene might reside in the smallest region of overlap.

P02.047
What management for the asymptomatic men carriers of *BRCA1* and *BRCA2* mutation? Inquest to the French oncogenetics centres.

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Objective: To define in the absence of guidelines, the management in France of asymptomatic men bearing a mutation of *BRCA1-2* gene.
Material and method: This multicentre study is a descriptive survey of practice. A questionnaire was addressed to the professionals working in the 90 French oncogenetics centres.
Results: We obtained the answers of 46 practitioners working in 58 centres. 100% of the responders offered this screening to determine the risk of transmission to the descent and 86% to offer a personal follow-up. This follow-up concerned for 94% the prostate cancer, for 68% the breast cancer, for 49% the pancreatic cancer, and for 12% the melanoma.
The screening of the prostate cancer was proposed mainly to the men bearing a *BRCA2* mutation and from the age of 40 years. It was based on the clinical examination and the prostate-specific antigen.
The screening of breast cancer was offered to the patient bearing a *BRCA2* mutation. It was proposed by self-palpation and/or medical clinical examination and started between the age of 30 and 50 years. Imagery was only realized in case of symptoms.
The screening of the pancreatic cancer was offered after the age of 40 years and by computed tomography and/or pancreatic MRI. It was mainly proposed in case of familial history of pancreatic cancer.
The general practitioner was considered to be the best to perform all these screenings.
Conclusion: These experts' opinions can help to establish recommendations for the management of the asymptomatic men carriers of *BRCA1-2* mutation.

P02.048
Rapid genetic counseling and testing in newly diagnosed breast cancer patients: Preliminary results of the TIME-trial

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Introduction: Female breast cancer patients carrying a BRCA1/2 mutation have an increased risk of second primary breast and ovarian tumors. Rapid genetic counseling and testing (RGCT) may aid in making informed decisions about therapeutic and preventive surgery and adjuvant treatment. Little is known about the effect of RGCT on treatment decisions and psychosocial health. We are currently carrying out a randomized controlled trial to investigate these issues.
Methods: Newly diagnosed breast cancer patients with minimally 10% risk of a BRCA1/2 mutation have been recruited from 12 Dutch hospitals. They were randomized to an intervention group (RGCT) or a usual care control group. Study outcomes include uptake of RGCT, surgery choice, cancer risk perception, cancer-specific distress, quality of life and decisional satisfaction. Assessments take place at study entry, and at 6 and 12 months follow-up.
Results: Between November 2008 and December 2010, 271 women were recruited and randomized to the intervention (n=184) or control (n=87) group. Currently, 106 women have completed all assessments (intervention group n=70). Of the 70 women in the intervention group, 63 chose DNA-testing (90%) and 40 (57%) opted for accelerated DNA test procedures: 22 (31%) for rapid testing (<4 weeks) and 18 (26%) for semi-rapid testing (4 weeks-4 months). Normally, test results are only available after 4 months. Eight (13%) patients were BRCA1/2 mutation carriers.
Conclusion: Preliminary results indicate high uptake of RGCT among high-risk breast cancer patients. Thus far, the majority of patients who are offered RGCT take advantage of this accelerated DNA-testing procedure.

P02.049
A case of breast cancer after radiation therapy for a skin haemangioma in childhood in a woman carrying a BRCA1 mutation

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It has been shown that radiation therapy, most of all during infancy, increases the risk of developing cancers later on. Here we present the case of a patient who received phosphorus ionizing therapy before the age of 1 for a skin haemangioma on the left breast. At the age of 25, the patient noticed a lump at the exact place where she received radiation therapy. At the age of 30, she was diagnosed with breast cancer. It was an infiltrating stage III ductal carcinoma, estrogen receptor negative, progesterone receptor negative and cerb2 negative, located exactly beneath the spot where she was treated while a child. Because of her young age when she was diagnosed, she was offered a genetic analysis of the *BRCA1* and *BRCA2* genes. A large deletion of 5 exons (8 to 13) of the *BRCA1* gene was found.
We are making the hypothesis that the radiation therapy she received during her infancy caused late toxicity that increased the risk she already had to develop breast cancer because of the mutation she carries on the *BRCA1* gene. We thus made a radio-induced and lymphocyte apoptosis test.
The patient herself is worried that her breast cancer was caused by the treatment she received for her haemangioma and therefore refused to receive radiation therapy and refuses to undergo breast x-rays for her follow-up.

P02.050
Brittle Cornea Syndrome in an Omani family with a novel mutation in *ZNF469* gene

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Brittle cornea syndrome (BCS-OMIM # 229200) is a rare autosomal recessive disorder that affects a variety of connective tissues with variable expressivity. The most dramatic feature of the disorder is the ocular phenotype presenting in 50% of cases with recurrent rupture of the thin and fragile cornea either spontaneously or secondary to minor trauma causing progressive visual loss and blindness. All affected individuals have blue sclera with many patients myopic. Secondary glaucoma and retinal detachments has rarely been reported. Systemic manifestations of other organs with connective tissues are common but less severe including skin elasticity, hyper mobility of joints with dislocation of large and small joints; kyphoscoliosis; dental anomalies; reduced bone mass density, congenital hearing loss and minor cardiac defects. BCS affects diverse ethnic groups with most patients reported from Middle East. We describe the clinical and morphologic features of affected individuals in a highly inbred Omani family affected with BCS and molecular confirmation of a homozygous c.1444delC mutation in the first exon of the *ZNF469* gene. This is the first case

report of an Omani family with BCS with a novel mutation in *ZNF469* gene. The ZNF469 protein has 30% homology to the helical parts of highly expressed corneal collagens, COL1A2, COL4A1 and COL1A1 and its proposed function is in the synthesis and/or organization of these collagen fibers. The differential diagnosis of BCS is the EDS type VI. Though the life expectancy of BCS patients is usually normal, affected patients usual have progressive visual loss despite protective measures.

P02.051
Search for neutropenia should be part of the first screening in patients with poikiloderma

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Poikiloderma occurs in a number of hereditary syndromes, the best known of which is Rothmund-Thomson syndrome (RTS). Differential diagnoses include Dyskeratosis congenita and Clericuzio-type poikiloderma with neutropenia (CPN). CPN is a rare autosomal-recessive genodermatosis characterized by poikiloderma, pachyonychia and chronic neutropenia, exhibiting phenotypic overlap with RTS. The clearest distinction seems to lie in the neutropenia with an increased susceptibility to infections. Mutations in the *RECQL4* gene observed in two third of RTS patients appear to be absent in CPN patients. Recently, mutations in the *C16orf57* gene has been identified in CPN patients. To date, the 33 reported patients with *C16orf57* mutations presented with neutropenia. In this study, we report on the clinical data and the *C16orf57* molecular analysis in 10 *RECQL4*-negative patients referred for syndromic poikiloderma. Other symptoms included photosensitivity (6/10), growth retardation (2/9), dysmorphism (6/10), dysplastic hair (7/10), nail dystrophy (2/10), bone abnormalities (1/10) and cataract (1/10). None had absent knee caps, gastrointestinal problems nor osteosarcoma. Two *C16orf57* heterozygous nonsense mutations (p.W81X and p.Y89X) were identified in one 5-year-old patient presenting with dental dysplasia, gingivitis, nail dystrophy, palmoplantar keratoderma, pachyonychia of the great toenails and generalized poikiloderma. No history of recurrent infections was reported. Previously undetected neutropenia was discovered after *C16orf57* molecular analysis. Neutropenia was absent in *C16orf57*-negative patients. Our report confirms that neutrophil count should be performed in all patients with poikiloderma to target the *C16orf57* gene sequencing analysis, prior to *RECQL4* study.

P02.052
Impaired vasoreactivity in CADASIL patients without significant disability

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Background and Purpose. CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) is a rare genetic disease caused by mutations in the NOTCH3 gene. A dysfunction in vasoreactivity has been proposed as early event in the pathogenesis of the disease. Aims of our study was to verify whether endothelium-dependent and -independent function is impaired in CADASIL patients compared to age-, gender-matched controls stratified by the presence of cardiovascular risk factors (CVRF).

Methods. We studied vasoreactivity in 49 CADASIL patients (30-65 years), 32 male (58%) without significant disability (Rankin scale score equal or less than 2) and 25 controls by a non invasive pletismographic method. Endothelium-dependent vasodilation was assessed by reactive hyperemia (FMD-PAT) while endothelium-independent vasoreactivity was studied by glyceryl trinitrate (GTN-PAT) administration.

Results. FMD-PAT scores were similar (1.88 [1.57-2.43] vs 2.08 [1.81-2.58], *P*=ns), while GTN-PAT values were significantly lower in CADASIL (1.54 [1.01-2.25] than controls 1.89 [1.61-2.59], *P*=0.041*)*. Among CADASIL patients, values below the 10 percentile of FMD-PAT scores in the control population were found in 17 (35%) , *P*=0.013. FMD-PAT and GTN-PAT values correlated significantly both in controls (rho=0.648 P<0.001) and CADASIL patients (rho=0.563 P<0.001)

Conclusions. The impaired vasoreactivity observed in our CADASIL patients highlights that both endothelial and smooth muscle functional alterations may occur already in pre symptomatic subjects. The improvement of vascular function could be a new target for pharmacological trials in the CADASIL population.

P02.053
Cantrell's Pentalogy with Complete Thoracoabdominal Ectopia Cordis and Asplenia - Expanding the Clinical Phenotype?

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Cantrell's Pentalogy (CP) is a rare, mainly sporadic, spectrum of congenital midline thoracoabdominal defects, including sternal defects, ventral diaphragmatic hernia, partial absence of the pericardium, supraumbilical abdominal wall defects, and congenital heart defects. The approximate incidence is 1 in 100000, with a 2:1 male predominance.

We report the case of a fetus affected by complete ectopia cordis, absence of the sternum and pericardium, defect of the ventral diaphragm, common aorticopulmonary trunk, cranio-facial dysmorfisms (microtia and bilateral cleft lip and palate) and asplenia without detected chromosomal anomalies on fetal karyotyping; thus leading to the diagnosis of CP with associated anomalies.

The severity of CP may range from incomplete to severe clinical phenotypes with the involvement of further organs/systems. Most of CP cases are sporadic and have been associated viral infections, maternal abuse of beta-aminopropionitrile, chlorine inhalation and numerical chromosomal aberrations.

Since the original Toyama's classification, a number of non-typical CP presentations have been reported (with a wide spectrum of multi-organ malformations). To our best knowledge, however, the association of CP with asplenia has never been previously reported.

P02.054
Cat-eye syndrome phenotype and extragonadal mature teratoma: an unusual case of dup 22q11.1q11.21

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We report the clinical and molecular findings of a 9 month old girl presented with a palpable midline neck mass, proved to be a extragonadal mature teratoma and also features of Cat-Eye Syndrome (CES). Cytogenetic analysis revealed a supernumerary marker chromosome which was identified by molecular cytogenetic analysis with fluorescence in situ hybridization (FISH) to derive from

chromosome 22. High resolution micro-array based comparative genomic hybridization (array-CGH) confirmed the marker and additionally clarified the size, breakpoints and gene content of the duplication [dup 22q11.1q11.21, 1.6 Mb; breakpoints: 15,438,946-17,041,773]. Cat-Eye syndrome is a disorder with a large variability, ranging from almost normal phenotype to severe malformations, but to our knowledge teratoma has not been previously reported. Also this is the first case of CES with well delineated breakpoints identified with high resolution array-CGH. Our findings extend the phenotypic spectrum of the 22q11.1-q11.2 duplication syndrome, pointing out specific dosage sensitive genes that might contribute to specific phenotypic features.

P02.055
CCR5 chemokine receptor polymorphism in HIV-1 patients from Western India

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Background: Entry of HIV-1 into target cells requires both CD4 and chemokine receptors. A 32-nucleotide deletion (?32) within the â-chemokine CCR5 gene has been described in HIV-1 subjects who remain uninfected. Our objective was to determine the frequency of ?32 deletion in CCR5 among HIV-1 infected patients and compare them with normal uninfected healthy individuals belonging to the same ethnic background. **Materials & Methods**: A total of 153 HIV-1 infected patients confirmed for their HIV-1 status were and a total of 68 normal Healthy individuals from the same ethnic background confirmed HIV-1 negative were studied. Genomic DNA from patients and controls were extracted and amplified for CCR5 gene segment using PCR. Amplified products were analyzed on 12% acrylamide gel, which yielded an 189bp fragment for wild type allele and 157bp fragment for the deleted (mutant) allele. **Results:** The results revealed that 7.19% were homozygous ,0.65% were heterozygous and the remaining 92.16% had no deletion for ?32 allele among the infected subjects.10.29% were homozygous ,0.00% were heterozygous and the remaining 89.71% had no deletion for ?32 allele among the control subjects. **Conclusion:** This study shows that the CCR5 allele frequency varies in different ethnic groups. Around 7.84% of the Patients have not been protected though they have the presence of the 32bp deletion.

P02.056
Congenital malformations and perinatal complications in CDG syndrome

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Congenital Disorders of Glycosylation is a growing group of more than 30 different types of inborn errors with multisystem involvement and central nervous system presentation. Supportive therapy and adequate counselling rely on early diagnosis of the underlying defect. Recently several patients were reported with CDG syndrome and perinatal complications.

We assessed 48 CDG patients from our patient cohort for the presence of prenatal and perinatal findings in various CDG forms. We included 29 children with a type I transferrine isoelectric focusing pattern: 6 patients with PMM2-CDG, 2 with ALG6-CDG, 1 female with DMP3-CDG, 7 children with ALG1-CDG, 12 patients with SRD5A3-CDG and 1 child with PMI-CDG. We evaluated 19 children demonstrating a type II TIEF pattern: 3 patients with COG7-CDG, 1 with B4GALT1-CDG, 12 patients with ATP6V0A2-CDG, and 3 with CDG-IIx.

We found maternal complications in 4 cases, including maternal eclampsia and reduced fetal movements. Prematurity/dysmaturity were observed in 9 cases. Microcephaly and neonatal seizures were common, especially in ALG1-CDG, COG7-CDG, ATP6V0A2-CDG. Dysmorphic features were present in almost all cases, however, mostly nonspecific, except for cutis laxa in ATP6V0A2-CDG. Ophthalmologic malformations and ichthyosis were characteristic in SRD5A3-CDG. Neonatal complications included feeding problems, microcephaly, hypotonia and neonatal bleeding. Three children diagnosed with either PMM2-CDG or ALG6-CDG had abnormal neonatal screening results for hypothyreoidism. Liver function tests were elevated in all neonatally evaluated cases.

Dysmaturity, microcephaly, congenital eye malformations and skin anomalies can be early signs of CDG syndrome. Screening for glycosylation disorders in these neonates is essential to obtain timely diagnosis.

P02.057***
Cerebellar malformations: clinical and genetic heterogeneity

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Cerebellar malformations (CM) are common structural brain abnormalities, which are neuroradiologically, clinically and genetically heterogeneous. Several autosomal recessive genes such as *RELN, VLDLR, SLOS, PMM2, TSEN54, etc* have been implicated in its causation, as well as some X-linked genes such as *OPHN1* and *CASK*. Chromosomal abnormalities have been described rarely. Access to genetic testing is limited. **Aim**: To investigate the role and prevalence of copy number variants in patients with CM. **Methods**: We performed CGH (comparative genomic hybridisation) on 120 patients with varied brain abnormalities, recruited through the Oxford Brain Abnormalities Research Group, using customized 4x180K Agilent technologies arrays. Twenty five of these patients presented with a CM. **Results**: 4/25 patients (16 %) were found to have a microarray abnormality. Three of the abnormalities were on the X chromosome (involving CASK and OPHN1) and the remaining was a homozygous deletion (involving NPHP1). The cerebellar phenotypes associated with these findings ranged from mild vermis hypoplasia to cerebellar hypoplasia with brainstem involvement. Clinically, ataxia and developmental delay were the most consistent findings, although developmental delay ranged from mild to severe. Other putative aberrations were identified, but have not been proven to be pathogenic as yet. **Conclusion**: CGH is a useful genetic test for determining the cause of CM. X-linked CM should be kept in mind due to its implication on reproductive risks. Furthermore, genotypic and phenotypic variability highlights the need for a targeted high throughput sequencing approach that can be used as a single diagnostic platform.

P02.058
Cardiofaciocutaneous syndrome (CFC) - a novel mutation and six new patients

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Cardiofaciocutaneous syndrome (CFC) is a rare genetic condition with sporadic occurrence. About 100 cases have been published, but this number seems to be underestimated. CFC is characterized by distinctive facial features, heart defects, ectodermal abnormalities and mental impairment. The syndrome is caused by gain-of-function mutations in four different genes BRAF, KRAS, mitogen-activated protein/extracellular signal-regulated kinases: MEK1 and MEK2.

We present six new patients with CFC syndrome, confirmed by molecular studies. We identified five different mutations. One of these mutations has not been reported in the literature (c.785A>C in BRAF gene). A patient with a novel mutation manifested typical dysmorphic features, ectodermal anomalies and a heart defect. He presented with only mild learning difficulties, contrary to the most patients with CFC syndrome, who are moderately or severely mentally retarded.

Clinical and molecular findings will be discussed hereby in relation to the patients previously reported in the literature.
The study was supported by the Polish Ministry of Science and Higher Education (grant Nr 0056/B/P01/2008/35) and CMHI (grant No 190/08).

P02.059
A CGH-Array study in non-syndromic (primary) autism disorder
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INTRODUCTION:
Autism is a neurodevelopmental disorder with a strong genetic basis in its etiology. Cytogenetic abnormalities have been detected in 5-10% of the patients with autism. Conventional karyotype analysis has revealed that chromosomal structural aberrations such as translocation, inversion, deletion, and duplication play a role in causing autism spectrum disorders (ASD). Unfortunately, there is not a single specific biological marker clearly responsible from autism. Array CGH is a molecular karyotyping method remarkable with fast analysis and highly sensitive diagnostic value.We aimed to use this technology to screen autistic patient's whole genome in order to investigate new dchromosomal abberations.
MATERIAL AND METHOD:
We isolated DNA samples of 35 primary autism patients between 0-18 years(median age:8.5).DNA samples which labelled according to the protocol, were hybridized with CytoSure Syndrome Plus(v2) 4x44K (Oxford Gene Technology, Oxford, UK) microchips and scanned with Agilent Microarray Scanner(Agilent Technologies, Palo Alto, CA). Obtained data were analyzed using Cytosure Analysis Software v.2.0.8 software(Oxford Gene Technology, Oxford, UK).
RESULTS:
We found 16p13.11 deletion in thirteen patients, 16p11.2 deletion in twelve patients, 1q21 deletion in ten patients, 2q21.1 deletion in eight patients, 2p21 deletion in seven patients, 8p23.1 deletions in seven patients.
CONCLUSION:
Accordig to these data, we can postulate that 16p13.11 region and related genes also represent a predisposition to autism in our patients. Verification of the data belonging to these regions, may provide important clues for gene expression and proteomics studies responsible from the pathogenesis of the disease and treatment strategies.

P02.060
Char Syndrome: A rare dysmorphic patient with new clinical findings
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Char syndrome is an extremely rare disorder, and exact prevalence of this syndrome has not been determined. Char syndrome is characterized by the triad of typical facial features, patent ductus arteriosus (PDA), and aplasia or hypoplasia of the middle phalanges of the fifth fingers. The patients with Char Syndrome have peculiar facial features incluiding flat midface, flat nasal bridge and broad
flat nasal tip, wide-set eyes, downslanting palpebral fissures, mild ptosis, short philtrum resulting in a triangular mouth, and thickened (patulous) everted lips. Our patients was born to a non-consangineous family after 37 week-old gestation under medical supervision because of oligohydramnios. According to our knowledge tracheomalacia, astigmatism and oligohydramnios is being published in Char Syndrome for the first time. New findings will be discussed and compared as well as other clinical findings with previously reported patients with Char Syndrome.

P02.061
Ectopic calcification in the Azores - Genetic aspects of DISH and chondrocalcinosis families
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Twelve families with early onset of calcium pyrophosphate dehydrate (CPPD) chondrocalcinosis (CC) and diffuse idiopathic skeletal hypersostosis (DISH) were identified in Terceira Island, the Azores, Portugal. The interest in this disorder results from its unusual and exuberant radiological characteristics, its disabling potential at a young age and the fact that it appears to be monogenic.
After clinical and radiological characterization, 92 individuals, from 12 unrelated families, were selected for a whole genome linkage study. DNA was extracted and HLA typing was carried out in probands and several family members; hereditary haemochromatosis mutations were screened and microsatellite amplification was performed using LMS V.2. Mendelian inheritance errors were checked using PEDCHEK. Parametric and non-parametric linkage analysis was performed using GENEHUNTER and MERLIN.
No association with HLA alleles was seen; haemochromatosis mutations were identified in 31 individuals from 9 families. Suggestive linkage to an area of chromosome 16 (16q12.1-16q22.1) was obtained in the whole genome analysis performed.
Although the molecular basis of this disorder has not been totally clarified, this study showed that these families were not associated to chromosomal areas previously linked to either DISH or CC. Through the analysis of pedigrees this disorder seems to be monogenic. Thus, the results of this study suggest the involvement of a possible new major gene in the aethiopathogenesis of this disorder.

P02.062
Case report: additional malformations in isochromosome 18p syndrome
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INTRODUCTION: Tetrasomy 18p resulting from an isochromosome 18p is a rare karyotype and is not well established phenotypically. To date, there are more than 40 patients described, mostly presenting mental retardation and minor features. CASE REPORT: A six month old baby girl was referred for genetic evaluation because of psychomotor retardation and dysmorphic features. She is the first child of young and healthy non-consanguineous parents. Familial history was negative. The pregnancy was complicated by preterm labor and threatened abortion. She was born by cesarean due to pelvic presentation. The clinical evaluation of the proposita showed hypotonia, a broad forehead, high hairline in front and back, oval facial shape, sparse hair and eyebrows, low-set and malformed ears, telecanthus, down-slanting palpebral fissures, convergent strabismus, infantile hemangioma in front, glabela and philtrum, small nose, microstomia, retrognathism, and proximally set thumbs. A cranial computed tomography scan showed abnormal enlargement of sulci and cistern around the brain, with predominance of frontal areas. Echocardiogram revealed interventricular communication with hemodynamic effect. The cytogenetic analysis with GTG-banding karyotype showed 47,XX,+mar. Based on dysmorphic features and cytogenetics characteristics, it was performed FISH with the centromeric probe chromosome 18, confirming the origin of the chromosome marker. Subtelomeric probe (RP11-324G2) detected isochromosome 18p; karyotype was defined as 47,XX,i(18)(p10). DISCUSSION: Despite of common dysmorphic signs described in tetrasomy 18p, this girl presents two major features which have not been described. Thus, this report contributes to the delineation of this chromosomal aberration.

P02.063
Clinical and cytogenetic findings in a girl with dup16 (q12.1q21)
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We describe a three year old girl admitted for evaluation because of speech delay, aggressive behaviour and dysmorphic facial features. Family history and pregnancy were unremarkable. Newborn period and early childhood were complicated with respiratory, gastrointestinal

and urinary tract infections. Associated structural anomalies of respiratory or urogenital tract as well as immune deficiency have been ruled out. Clinical examination revealed mild dysmorphic features: dysplastic ears, lower forehead, palpebral fissures slant down, hypoplastic supra-orbital ridges, blepharophimosis, epicanthic folds, flat nasal bridge, bulbous nose, long philtrum, thin upper lip, small mouth, and short neck. Marked lumbar lordosis and broad thorax were observed. Hands and feet were small with thick fingers. Audiometric findings were normal. Ophthalmologic examination revealed left side strabismus and hypetmetropy. Ultrasound examination of abdomen and pelvis was normal as well as EEG and CT of the brain. Routine cytogenetic chromosome analysis showed additional material on chromosome 16. FISH analysis with WCP-16 probe revealed that this material originates from chromosome 16. Microarray analysis with a ≈75 kb resolution showed a gain on the long arm of chromosome 16 at bands q12.1 through q21, which is approximately 9.92 Mb in size. To the best of our knowledge, this is a first description of a patient with dup16 (q12.1q21). The possible influence of the triple dosage of genes located in this region on clinical presentation of our patient is discussed.

P02.064
Chromosome instability syndrome with congenital heart disease.

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Clinical case: Female patient, 4 y.o., came under our observation with congenital heart disease (CHF, atrial septum defect) required surgical correction. During clinical examination additional phenotypic features (mental retardation, hidden facial cleft, epicanthi, clinodactyly) were found. She had verbal development delay, and normal hematological parameters.

Cytogenetic analysis was performed to identify chromosome aberrations. Two consecutive karyotyping was carried out to confirm primary finding. Lymphocytes from peripheral blood samples were obtained from 72-h mitogen stimulated cultures, prepared according to standard cytogenetic procedures. The GTG-banding analysis revealed normal female karyotype, 46, XX, with common chromosome instability in 30-40% percent of cells.

Discussion: Patient was discharged after successful surgical correction of CHD with chromosome instability syndrome diagnosis. There were no additional features usually associated with chromosomal breakage syndrome. The chromosome instability syndromes are group of conditions associated with chromosomal instability and breakage, and high risk of malignancy. In this case chromosome instability can modulate the risk of lympho-proliferative disease. The mean age of leukemia manifestation is 5-6 years old. We did unmask high risk of hematological complications in patient with primary cardio surgical problem.

Conclusion: Routine karyotyping performed for CHD patients can reveal chromosomal aberration in about 20%. We suppose this analysis has to be done routinely for such a group of patients even after neonatal period.

P02.065
Genetic polymorphism of human Y chromosome and risk factors for cardiovascular diseases: a study in WOBASZ cohort

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Genetic variants of Y chromosome predispose to hypertension in rodents whereas in humans the evidence is conflicting. Our purpose was to study the distribution of a panel of Y chromosome markers in a cohort from a cross-sectional population based study on prevalence of cardiovascular risk factors in Poland (WOABSZ study). The HindIII and YAP Y chromosome variants previously shown to influence blood pressure, lipid traits or height were typed in 3026 and 2783 samples. In addition 4 subgroups (N~100 each) representing extremes of LDL concentration or blood pressure (BP) were typed for a panel of SNPs defining main Y-chromosome haplogroups and 19 STRs. The HindIII or YAP polymorphism were not associated with any of the studied traits. Extended analyses suggested a link between haplogroup N3* and lower mean diastolic BP (P=0.009) but this finding was not replicated when a larger group of subjects (N=1077) was analyzed (P=0.69, a trend in opposite direction). Analysis of STRs did not show statistically significant differences apart from the DYS385II marker whose distribution suggested effects on both BP (P=0.015) and LDL (P=0.034) concentration. In conclusion, our study shows that the widely studied Y chromosome HindIII variant and the YAP variant do not influence blood pressure, lipid traits or anthropological characteristic. Although we cannot exclude an association between DYS385II and BP/LDL concentration our results do not support a major role of Y chromosome genetic variation as defined by major Y chromosome haplogroups in determining cardiovascular risk in Poles.

P02.066
Orofacial phenotype of van der Woude and Poplyteal Pterygium Syndrome patients mutated for IRF6

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Van der Woude syndrome (VWS, OMIM #119300) is a dominantly inherited developmental disorder characterized by pits and/or conical elevations of the lower lip, cleft lip and/or palate, and hypodontia. Popliteal pterygium syndrome (PPS, OMIM #119500) shares the clinical features of VWS, with the addition of other signs such as popliteal webs (pterygia), synechiae connecting the upper and lower jaws, ankyloblepharon, syndactyly and genital anomalies.

Since many signs occur in the orofacial region and the penetrance is incomplete, it is of great interest to better characterize VWS/PPS patients to see if an oral phenotype-genotype correlation exists. For this, nineteen families (17 VWS + 2 PPS) from Trousseau hospital (Paris) were seen by a multidisciplinary staff, focusing on the orofacial phenotype. Panoramic imaging were used to check for dental agenesis. The coding exons of the IRF6 gene, including intron-exon boundaries, were sequenced, and Multiplex Ligation-dependent Probe Amplification was performed to test for intragenic deletions or amplifications. We identified a mutation in IRF6 in 82% (14/17) of VWS and 100% (2/2) of PPS families. Affected individuals had clefts (79%), lower lip pits (78%), dental agenesis (72%), and abnormal dental morphology (60%). The upper lateral incisors and the second upper premolars were the most affected.

This prospective study demonstrates the high frequency of dental anomalies in VWS/PPS patients, affecting mostly the upper lateral incisors and the second upper permanent premolars. This may be a useful clinical clue for correct diagnosis, as lips pits are not always present.

P02.067
Duplication of chromosome 15q22 in patient with severe dysmorphic features

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We report on one-year-old girl with chromosome 15 duplication and severe dysmorphic features. Our patient is the second child of uncomplicated pregnancy with high risk of biochemical testing on second trimester from non-consanguineous parents. The girl's phenotype is characterised by microcephaly, ocular hypertelorism, downslanting palpebral fissures, intensive blue sclera, prominent nasal bridge with periorbital fullness, short triangular philtrum, microstomia, partial cleft hard and soft palate, preauricular sinuses, short neck, expressed hypertrichosis on the whole body, pilonidal

sinus, psychomotor retardation and grow delay.
Patient was karyotyped and a normal female karyotype was detected. We used subtelomeric FISH (ToTelVysionTM) for search of subtelomeric microdeletions/microduplications for the possible cause of intellectual disability and congenital malformations of the patient. Analysis of FISH signals of chromosome 15 indicated additional control signal (15q22 LSI PML) on terminal arm of other chromosome of undetermined origin. We suggest that duplicated region of 15q22 may be an appreciable cause of various severe dysmorphic features of the patient. Array CGH analysis for verification and specification of this duplicated region is under the investigation.

P02.068
Cholestasis in patients with Cockayne syndrome and suggested modified criteria for clinical diagnosis

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Cockayne syndrome (CS) is a rare autosomal recessive neurodegenerative disease characterized by growth failure; brain dysmyelination, cutaneous photosensitivity; pigmentary retinopathy, cataract, sensorineural hearing loss and hepatomegaly. To the best of our knowledge, cholestatic liver disease was not previously reported in these patients.
Aim: To highlight the presence of cholestasis and liver dysfunction in this group of patients and to suggest modified criteria for clinical diagnosis.
Methods: The study included nine patients with CS from four different families (five males and four females) in which CS was suspected clinically. For all patients cytogenetic analysis revealed spontaneous chromosomal breakage that was increased after mitomycin C treatment. Diagnosis was confirmed by DNA repair assay.
Results: Seven of these patients had evident liver affection ranging from mild elevation in liver enzymes to cholestatic liver disease and liver cell failure. The attacks were recurrent in two patients and were sometimes preceded by infection. These attacks may lead to deterioration of liver condition or neurological symptoms or it may end in liver cell failure that may recover completely or it may end in death.
Conclusion: liver disease could be considered common in Egyptian patients with CS with the cholestatic form being the most evident. Chromosomal breakage study and positive family history should be included as major criteria for clinical diagnosis of CS especially in a population like ours whereas consanguineous marriage is very high and molecular testing and UV sensitivity tests are considered unaffordable.

P02.069
Cleft palate and bilateral congenital cataract: a familial observation

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We report the observation of a family with a rare association of congenital bilateral cataract (**CBC**) and cleft palate. The family pedigree has unveiled abnormalities over 4 generations, affecting 21 persons with CBC +/- cleft palate. The transmission seems autosomal dominant.
Clinical history
We report 4 brothers and sisters (2G-2B), followed in pediatrics surgery, presenting a CBC associated to a cleft palate:
-Case 1: Propositus, girl, CBC, Pierre Robin (Sub-mucous cleft (**SMC**), palate fistula), ptosis and growth delay (-2DS)
-Case 2: girl, CBC, Pierre Robin (cleft of the soft-palate), strabism, facial dysmorphism with mongoloid palpebral fissures and large nose
-Case 3: boy, CBC, SMC and bifid uvula, facial dysmorphism with mongoloid palpebral fissures, large nose and language delay.
-Case 4: boy, CBC, bifid uvula
-There is a fifth healthy female sibling.
The mother has facial dysmorphism, CBC, SMC, clinodactyly and scoliosis.
The propositus' karyotype was normal. Array-CGH analysis showed an interstitial amplification in Xp21.1, found in: the mother, propositus (case 1), but also in the healthy girl and not found in Case 2.
Discussion
The clinical association of cleft palate and ophthalmic abnormalities is not rare and has been reported in over 50 syndromes, but there are few familial observations with 4 affected generations.
In conclusion
Based on the genetic-clinical discordance we can wonder about the deleterious impact of the Xp21.1 amplification that might be a copy number polymorphism. It seems relevant to further study the underlying genetic mechanisms of this clinical association.

P02.070
Coffin-Lowry syndrome: report of 3 novel mutations in RPS6KA3 gene

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Coffin-Lowry syndrome (CLS) is an X-linked semidominant disorder characterized in male patients by mental retardation, facial dysmorphism and skeletal malformations. Affected females usually show variable features. The only known gene associated with CLS is RPS6KA3 that encodes the serine/threonine kinase. Loss-of-function mutations are distributed throughout the gene. To date over 140 different mutations have been identified.
Here, we present 3 patients (two males and female) with CLS caused by novel RPS6KA3 gene mutations. The molecular analysis was performed by single-strand conformation polymorphism (SSCP) followed by sequencing of the gene.
The first male patient (1y3m) presented with hypotonia, developmental delay, and typical dysmorphic features: hypertelorism, frontal bossing, down-lanting palpebral fissures, anteverted nares, thick lips, broad and tapering fingers. Molecular analysis revealed a substitution mutation c.1443+3A>C. His two sisters and mother had dysmorphic features suggesting CLS carrier status.
The second male patient (1y1m) presented with hypotonia, developmental delay and facial anomalies (hypertelorism, down-slanting palpebral fissures, broad nose, everted lower lip). His hands were fleshy with simian creases and tapering fingers. Molecular testing showed a deletion c.889_890 del AG. Mother revealed tapering fingers as her only feature.
The third proband, female (2y4m), was referred for genetic evaluation because of developmental delay. She had dysmorphic features: hypertelorism, down-slanting palpebral fissures, frontal bossing, soft hands with tapering fingers and persistent anterior fontanelle. Molecular analysis revealed a substitution c.823G>T.
All mutations change the function of the serine/threonine kinase which with typical symptoms of CLS in patients confirm the pathogenic character of changes.

P02.071
Frequency of 22q11.2 microdeletion in children with congenital heart defects in Western Poland

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The 22q11.2 microdeletion syndrome refers to congenital abnormalities, including heart defects and facial dysmorphy, thymic hypoplasia, cleft palate and hypocalcaemia. Microdeletion within chromosomal region 22q11.2 constitutes the molecular basis of this syndrome. The aim of

this study was to determine the frequency of 22q11.2 microdeletion in 87 children suffering from a congenital heart defect (conotruncal or non-conotruncal) coexisting with at least one additional 22q11.2DS feature and to carry out 22q11.2 microdeletion testing of the deleted children's parents. We also attempted to identify the most frequent heart defects in both groups and phenotypic traits of patients with microdeletion to determine selection criteria for at risk patients.
The analysis of microdeletions was conducted using fluorescence *in situ* hybridization (FISH) with molecular probe specific to the *HIRA* region at 22q11.
Microdeletions of 22q11.2 region were detected in 13 children (14.94% of the examined group). Microdeletion of 22q11.2 occurred in 20% and 11.54% of the conotruncal and non-conotruncal groups respectively. Tetralogy of Fallot was the most frequent heart defect in the first group of children with 22q11.2 microdeletion, while ventricular septal defect and atrial septal defect/ventricular septal defect were most frequent in the second group. The microdeletion was also detected in one of the parents of the deleted child (6.25%) without congenital heart defect, but with slight dysmorphism.
Our study suggests that screening for 22q11.2 microdeletion should be performed in children with conotruncal and non-conotruncal heart defects and with at least one typical feature of 22q11.2DS as well as in the deleted children's parents.

P02.072
Molecular and clinical characterization of Estonian congenital heart disease patients with septal defects: screening of NKX2.5, GATA4 and TBX5 genes.

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Congenital heart disease (CHD) is one of the most common birth defects (0.5-1% live births), but the genetic causes remain largely unknown. The most frequent forms of the CHD are the septal defects (SD). In earlier studies have been referred that mutations in genes *NKX2.5, GATA4, TBX5* may be associated with SD. The aim of our study was the clinical and molecular characterization of patients with CHD.
Study group consisted of patients who were referred to genetic counseling during 1990-2010 due to CHD, which included SD. SD was isolated or one part of combined malformation. *NKX2.5*, *GATA4* and *TBX5* coding exons and intron-exon boundaries were analysed by PCR amplification and sequencing. In the study group there were 22 patients (nine familiar cases): 15 index cases (8 females, 7 males) and 7 family members. In most of the cases there were combined CHD, which included SD (atrio- and/or ventriculo-SD). During analyses of *NKX2.5* found a new mutation (C.178 G>C, p.Glu60Gln) in a patient with Fallot' tetrad and 60% of probands had a known synonymous mutation E21E (rs2277923) in exon 1. In *GATA4* gene we found a known mutation c.1647 A>G, p.S377G in the exon 6 in one affected family member and in *TBX5* gene we found a frequent intronic SNP rs2236017 in 53% of index cases. Analyzed transcription factor genes *NKX2.5, GATA4* and *TBX5* probably have modest effect on the formation of SD in CHD. The research of the regulatory variants may add some more information. Supported by GARMP6573 and GARLA6808

P02.073
Congenital heart defects in association with dextrocardia

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Dextrocardia is a congenital anomaly in wich the heart is positioned abnormally within the right side of the chest with the apex pointing to the right. Sometimes it is associated with situs inversus totalis or heterotaxy. The prevalence and the type of associated heart defects are variable.
We have analysed the prevalence and types of congenital heart defects in 16 children with dextrocardia recorded in the files of Iasi Medical Genetics Center. There were 5 girls and 11 boys; 10 had associated situs inversus totalis, 5 had situs solitus and 1 heterotaxy. Heart defects were present in 10 children (62.5%) - septal defect (27.7%), Fallot tetralogy (12.5%), transposition of great arteries (12.5%), aortic stenosis (6.25%), single ventricle(6.25%), left ventricle hypoplasia (6.25%), anomalous venous return (6.25%) and mitral valve prolapse (6.25%). The prevalence of heart defects was similar in boys (63.63%) and girls (60%). Complex heart defects were present only in males and more frequent in situs solitus and heterotaxy group (50%) than in situs inversus group (40%). In the literature common atrium, septal defects, pulmonic stenosis, transposition of great arteries and right aortic arch are the most frequent congenital heart defects associated with dextrocardia. In conclusion we present a study of 16 cases with dextrocardia, 62.5% of them having heart defects. Cardiac defects are commonly associated with dextrocardia and males are more severely affected than females. When dextrocardia occurs in the context of situs solitus or heterotaxy, the risk of associated congenital heart defects is higher.

P02.074
Low level mosaicism detected by array CGH in congenital malformations syndrome

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Congenital malformations present at delivery of an infant are due to genetic or non genetic factors and occur in 15-20% of stillborn children. Most can be diagnosed prenatally by ultrasound examination, but some can only be diagnosed after birth. Seven to 10% of infants with abnormal phenotype have numerical or structural chromosomal abnormalities that require identification for accurate diagnosis and genetic counseling. Molecular-cytogenetic and array-based techniques have enabled screening at higher resolution for congenital anomalies that result from genomic imbalances. We have examined four children with congenital anomalies, with or without mental retardation, of unclear etiology. In one child, we detected a deletion (about 28 Mb) of the region 18q21.1-18q23, in mosaic form. This abnormality was missed in a routine cyto genetic examination. We detected different polymorphic copy number variations (CNVs) in the other children. After repeating the cytogenetic analysis in high number of metaphases, we observed the deletion cytogenetically in 17% of the cells. We conclude that array-based comparative genomic hybridization (CGH) is a powerful diagnostic tool for the detection of low level mosaicism.

P02.075
Congenital anomalies and legal abortion in Iran

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Multiple congenital anomalies are common disorders that maybe occur in fetus due to acquired, genetical or multifactorial causes. Every time pregnant women or fetuses are seriously suffering from critical disorders can lead to infirmity, and the legal abortion is recommended. This is done based on forensic permission in Iran.
This case was referred to genetic counseling center for evaluation of fetal health. Some anomalies were found by sonography including intra uterine growth retardation, microcephaly, and club foot and finger anomalies. The pregnancy was terminated after three genetical and embryological consultations based on forensic ordinance. The proband was gravid 3 and the result of consanguineous marriage. Two previous pregnancies led to neonatal death following to congenital heart disease. Two other unknown cases of multiple congenital anomalies, one case of congenital heart disease, one case of mental retardation, one case of visual loss and two cases of hearing loss were seen in familial pedigree. In proband, the other fetal clinical manifestations

consisted of cleft palate, short and webbed neck, micrognathia, simian crease, clinodactyly, ectrodactyly, lung hypoplasia and joint stiffness. The search of signs revealed several autosomal recessive differential diagnosis such as Smith-Lemli- Opits syndrome type II, and Fryns syndrome. Bioethics which is the most important concept in medical evaluation and intervention is affected by the law and religion. There are important conflicts in principle branches of Islam. Any disorder which can lead to infirmity or critical condition for pregnant woman is considerable in legal medicine.

P02.076
Pediatric intestinal cancer and polyposis due to bi-allelic *PMS2* mutations: case series, review and follow-up guidelines

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Bi-allelic germline mutations of one of the DNA mismatch repair genes, so far predominantly found in *PMS2*, cause constitutional MMR-deficiency syndrome. This rare disorder is characterized by pediatric intestinal cancer and other malignancies. We report the clinical, immunohistochemical, and genetic characterization of four families with bi-allelic germline *PMS2* mutations. We present an overview of published gastrointestinal manifestations of CMMR-D syndrome and propose recommendations for gastro-intestinal screening.

The first proband developed a cerebral angiosarcoma at age 2 and two colorectal adenomas at age 7. Genetic testing identified a complete *PMS2* gene deletion and a frameshift c.736_741delinsTGTGTGTGAAG (p.Pro246CysfsX3) mutation. In the second family, both the proband and her brother had multiple intestinal adenomas, initially wrongly diagnosed as familial adenomatous polyposis. A splice site c.2174+1G>A, and a missense c.137G>T (p.Ser46Ile) mutation in *PMS2* were identified. The third patient was diagnosed with multiple colorectal adenomas at age 11; he developed a high-grade dysplastic colorectal adenocarcinoma at age 21. Two intragenic *PMS2* deletions were found. The fourth proband developed a cerebral anaplastic ganglioma at age 9 and a high-grade dysplastic colorectal adenoma at age 10 and carries a homozygous c.2174+1G>A mutation.

Tumors of all patients showed microsatellite instability and/or loss of PMS2 expression.

Our findings show the association between bi-allelic germline *PMS2* mutations and severe childhood-onset gastrointestinal manifestations, and support the notion that patients with early-onset gastrointestinal adenomas and cancer should be investigated for CMMR-D syndrome. We recommend yearly follow-up with colonoscopy from age 6 and simultaneous video-capsule small bowel enteroscopy from age 8.

P02.077
Multi-Exon Deletion of *SMC1A* in a Severely Affected Female with Cornelia de Lange Syndrome

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Cornelia de Lange Syndrome (CdLS) is a genetically heterogeneous disorder characterized by dysmorphic facial features, cleft palate, limb defects, growth retardation and developmental delay. Approximately 60% of patients with CdLS have an identifiable mutation in the *NIPBL* gene at 5p13.2. Recently, an X-linked form of CdLS with milder phenotype was attributed to mutation of the structural maintenance of chromosomes 1A gene (*SMC1A*) at Xp11.22. Only a handful of male CdLS patients with mutations in *SMC1A* are known. The phenotype in female carriers is limited to minor developmental and cognitive deficiency. To date, all mutations identified in *SMC1A* are missense or small in-frame deletions that likely have a dominant negative effect. We report a Turner mosaic female with a severe form of CdLS who presented with growth and mental retardation, multiple congenital anomalies and facial dysmorphism. Array CGH identified a novel deletion of *SMC1A* spanning multiple exons which suggested a possible loss-of-function effect. Additional molecular studies were initiated to more precisely define the deletion boundaries and the effect of this genetic alteration. Expression of a truncated RNA message was detected in lymphocytes. Sequencing of both genomic and cDNA demonstrated an 8,152 base pair deletion of genomic DNA from exon 13 to intron 16. Although the loss-of-function effects of this deletion cannot be excluded, the resulting mRNA remains in-frame suggesting a dominant negative effect. We hypothesize that the size of this deletion compared to previously reported mutations and the presence of mosaic monosomy X likely contributes to the severity of her phenotype.

P02.078
HRAS mutation analysis in Polish patients with Costello syndrome

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Costello syndrome (CS) is a rare congenital disorder with multiple anomalies including characteristic dysmorphic craniofacial features, short stature, failure to thrive, developmental delay, skin anomalies, cardiac and musculoskeletal defects and an increased risk of malignancy (about 10-15%). It has been demonstrated that *de novo* missense mutations in the proto-oncogene *HRAS* resulting in increased activation of Ras-MAPK pathway cause Costello syndrome. We present a group of five unrelated Polish patients with clinical diagnosis of CS. Mutation analysis of *HRAS* gene revealed heterozygous missense substitutions affecting aminoacid 12 of the protein product: c.34G>A (p.G12S) in two and c.35G>C (p.G12A) in three patients. Analysis of the available parental DNA confirmed four of the mutations as *de novo* cases. The evaluated group represented relatively homogenous Costello phenotype with coarse facial features, feeding difficulties and redundant skin with hyperpigmentation and deep plantar creases on hands and feet. Neonatal macrocephaly was present in all of the patients, as well as developmental delay, hypotonia and cardiac defects (cardiomyopathy, arrhythmia or pulmonary stenosis). Interestingly, in one study case the coexistence of Turner and Costello syndrome was observed. Our results deliver the first important information on the molecular basis of Costello syndrome in Polish population. They indicate certain heterozygous missense mutations in *HRAS* to be responsible for CS cases worldwide and confirm that the molecular analysis of *HRAS* provide a reliable diagnostic test for Polish patients diagnosed with Costello syndrome. The research was financed by MNiSW Project no. PB 0056/B/P01/2008/35 and CMHI Project no. 190/08.

P02.079
Craniofacial indices as a tool in evaluation dysmorphological features in parents with cleft children

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Introduction. There are many genetic diseases associated with abnormal craniofacial morphology. Comparison of craniofacial indices in family members of patients with clefts and healthy individuals can be used to evaluate dysmorphological features of the head and face. **Objective.** To ascertain whether craniofacial indices could be used in evaluation dysmorphological features**. Materials and methods.** The subjects of our study were parents of children with a clefts, and clinically healthy individuals. Eight measurements were used in calculation of the indices. **Results.** Five craniofacial indices were calculated separately for males and females. Results are presented in the table1 (for males) and table 2 (for females).

Table 1

	Clefts (N = 57)	Control (N = 39)				
Index	**Mean**	**SD**	**Mean**	**SD**	**MD**	**p**
Cephalic index	0,82	0,04	0,80	0,06	0,02	0,090
Facial index	0,85	0,06	0,94	0,10	-0,09	0,001*
Intercanthal index	0,33	0,03	0,27	0,03	0,06	0,001*
Upper face index (R)	0,94	0,12	0,95	0,03	-0,01	0,577
Upper face index (L)	0,96	0,03	0,96	0,03	0,00	0,954

Table 2						
	Clefts (N = 67)	Control (N = 38)				
Index	**Mean**	**SD**	**Mean**	**SD**	**MD**	**p**
Cephalic index	0,82	0,04	0,80	0,04	0,02	0,010*
Facial index	0,82	0,05	0,97	0,08	-0,14	0,001*
Intercanthal index	0,34	0,03	0,26	0,02	0,08	0,001*
Upper face index (R)	0,97	0,03	0,97	0,03	0,00	0,890
Upper face index (L)	0,97	0,03	0,98	0,03	0,00	0,405

* Statistically significant(p<0,05)

Conclusions. Basic craniofacial indices show differences between 2 study groups and therefore could be useful in evaluation craniofacial dysmorphology.

P02.080
A novel de novo mutation within *EFNB1* gene in a young girl with Craniofrontonasal syndrome

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Craniofrontonasal syndrome (CFNS) is mainly characterized by frontonasal dysplasia, telorbitism, broad nasal root and frequently a bifid nose and coronal craniosynostosis. CFNS is an X-linked disorder with an unusual pattern of inheritance, as heterozygous females are more severely affected than hemizygous males. The CFNS-causing gene is EFNB1, localized in the border region of chromosome Xq12 and Xq13.1, encoding for protein ephrin-B1. Ephrin-B1 belongs to the B-subclass of ligands of Eph receptors (receptor tyrosine kinases). Eph/ephrin signaling is important for the developmental processes of the embryo, including skeletal and craniofacial development. Here we aim to investigate the underlying genetic defect of a young girl with CFNS. The patient underwent surgical correction of her craniofacial deformities. Genetic analysis was carried out by Polymerase Chain Reaction (PCR). Products of exon 2 of EFNB1 gene were sequenced as well as digested with BpmI enzyme. A novel de novo missense mutation 373G>A was identified within EFNB1 gene, leading to the replacement of Glutamic acid at amino acid position 125 with Lysine. The replacement of Glu125 with Lys, which lies within the G-H loop, part of the dimerization ligand-receptor interface, is expected to disrupt the interaction between Eph receptor and ephrin B1 ligand, thus leading to CFNS. Missense mutations in exon 2 of *EFNB1* gene in patients with CFNS leading to aminoacid changes within the dimerization ligand-receptor interface have been described elsewhere.

P02.081
An atypical 5p deletion, involving part of the critical region for Cri-du-chat syndrome, in a girl with mild mental retardation

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Cri-du-chat syndrome results from a deletion of variable size on the short arm of chromosome 5. The chromosomal region implicated in the syndrome has been mapped to 5p15.1p15.3. Main clinical features are a high-pitched cat-like cry, distinct facial features, microcephaly and severe psychomotor and mental retardation. The phenotype varies depending on the size and location of the deletion. The critical regions for the typical cry and the mental retardation have been mapped to 5p15.3 and 5p15.2, respectively. *SEMA5A* and *CTNND2* located within the 5p15.2 critical interval have been suggested as candidate genes for the mental retardation. *CTNND2* is highly expressed in the brain, and is implicated in neuronal function. Furthermore, *CTNND2* knock out mice show severe cognitive dysfunction, and a correlation between deletion of *CTNND2* and severe mental retardation in humans has been reported. We report on a patient with a 3.2 Mb deletion of 5p15.2p15.1 (chr5:11902869-15234290, hg19) that displays mild mental retardation and developmental delay, but no dysmorphic features. Her deletion involves the genes *CTNND2*, *TAG*, *DNAH5*, *TRIO*, *FAM105A*, *FAM105B* and *ANKH*. We suggest that haploinsufficiency of *CTNND2* causes the mild mental retardation in our patient, and that other genes in the region contribute the significantly more severe cognitive impairments frequently seen in patients with Cri-du-chat syndrome.

P02.082
A novel mutation in HLXB9 gene in Currarino syndrome in an adult man: a case report

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The Currarino syndrome (CS; OMIM 176450) has been described as a triad of partial sacral agenesis with intact first sacral vertebra (sickle-shaped sacrum), presacral mass, and anorectal malformations. The sacral agenesis in CS is typically incomplete and only involves vertebrae S2-S5, a sacral anomaly distinct to this syndrome CS exhibits variable expressivity, reduced penetrance and remarkable variability of objective findings and subjective complaints, with a clinical spectrum ranging from anomalies localised only to the caudal region to those involving complex malformations of other organ systems including the kidneys, brain and spinal cord.

Currarino syndrome is autosomal dominant inheritance and has been associated with mutations of HLXB9, a homeobox gene that maps to chromosome 7q36. The prevalence is unknown and presentation in adulthood is extremely uncommon.

Here, we present a case of a 36-year old man with Currarino triad. He was diagnosed of CS after he suffered a motorbike accident. Until that moment, he presented chronic constipation. Mega-rectosigmoides and partial sacral agenesis was confirmed on X-ray and IRM. We performed direct sequencing for HLXB9 gen. We identified one novel mutation, c.264C>A, p. C88X.

The presence of variable phenotypes suggests DNA alterations in HLXB9 noncoding regions and/or in other genes encoding HLXB9 regulatory molecules or protein partners.

P02.083
Duplication of 11p with clinical features of Costello Syndrome and cutis laxa

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Cutis laxa (CL) is a disorder of wrinkled, abundant skin with abnormal elasticity. Several genetic defects have been elucidated to cause cutis laxa, still, the etiology in the majority of cases remains unknown. We describe a CL patient with duplication of the short arm of chromosome 11. Our patient had birth parameters at 90 percentiles, cutis laxa, cleft lip, several dysmorphic features, macroglossia, deep palmar/plantar creases, abnormal hair growth and hypotonia. She was diagnosed with hyperinsulinism, Meckel diverticulum, umbilical and inguinal hernias, ventricular septum defect, aqueductal stenosis feeding difficulties and developmental delay. Histology of skin showed severe abnormalities of elastin fibers. Genetic analysis revealed a duplication of 11p of paternal origin, which is associated with Beckwith Wiedemann syndrome (BWS). Many features in our patient are explained by the diagnosis of BWS, except for cutis laxa. Several clinical findings in our patient, including extensive skin wrinkling, could, however, also fit into the diagnostic criteria of CS. Intriguingly, the *HRAS* gene, in which gain-of-function mutations cause CS, is also located on 11p15.5. QPCR analysis in our patient showed an increase in *HRAS* expression in fibroblasts. Based on this genetic dosage effect and the observation of similar phenotypes in other 11p duplication cases we suggest that cutis laxa and the clinical features comparable to those of Costello Syndrome in our patient, occurred due to duplication of *HRAS*.

P02.084
Cytochrome P450 2C19 genetic polymorphisms and symptoms of gastroesophageal reflux disease.

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Background: Gastroesophageal reflux disease is a common disease worldwide and has typical and atypical symptoms such as heartburn and hoarseness.Cytochrome p450 (CYPs) enzymes, located mostly in the liver, are responsible for the metabolism of omeprazole. Cytochrome P450 2C19 (CYP2C19) gene has several polymorphisms, the most common of which are the CYP2C19* 2 and CYP2C19* 3 alleles.These two polymorphisms both decrease enzyme activity. This study was performed to evaluate the influences of two common CYP2C19 polymorphisms on the frequencies of typical and atypical symptoms in the Iranian patients with gastroesophageal reflux disease.
Methods: One hundred and sixteen patients with gastroesophageal reflux disease were enrolled in the study. A gastroesophageal reflux questionnaire was completed for each patient by direct interview. It was comprised of information such as age, sex, BMI and symptoms of disease. A volume of 2 ml of venous blood was sampled from each subject. CYP2C19 genetic polymorphisms were detected using the PCR-RFLP method.
Results: There was an association between the frequencies of some atypical gastroesophageal reflux disease symptoms such as belching, anorexia, chest pain and hoarseness and CYP2C19 polymorphism, as the frequencies of these symptoms were higher in the variant CYP2C19 genotype (P<0.040).
Conclusion: CYP2C19 genotype might influence the appearances of some atypical symptoms in the patients with gastroesophageal reflux disease.

P02.085
Developmental delay and facial dysmorphism in a girl with a 9.5Mb de novo interstitial deletion of 12q: further delineation of chromosome 12q15-q21 deletion syndrome

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Mental retardation, as isolated symptom or as part of syndrome, is a common condition affecting 2-3% of individuals worldwide. Chromosomal rearrangements identified by karyotype or array-based comparative genomic hybridization (CGH-arrays), account for about 15-20% of mental retardation etiologies. Interstitial deletions of the long arm of chromosome 12 are rarely described in the literature.
Here, we report on a 4-year-old girl presenting with a deletion involving the 12q15-q21.31 region. Her clinical features include microcephaly at birth, developmental and speech delay, growth retardation with progressive macrocephaly, facial dysmorphism, strabismus and small joints hyperlaxity.
A 9.5 Mb deletion was detected by standard karyotype and confirmed by CGH-array. This rearrangement was not found in the parents, consistent with a de novo occurrence. The deleted region involves 27 genes, 3 of them being hyperexpressed in the brain.
To our knowledge, only 4 other cases of deletion encompassing the 12q15-q21 region are reported in the literature. All patients had developmental delay and mental retardation. Most of them presented with microcephaly at birth, intra-uterine and postnatal growth delay, cardiac malformations and common facial dysmorphism (high forehead, broad nasal bridge, low-set ears, arched or cleft palate, and micrognathia).
Description of additional patients in the future will help to refine genotype-phenotype correlations in this chromosomal region, improving genetic counseling for pre- and postnatal 12q deletion cases.

P02.086
An emerging phenotype of microdeletion Xq24 involving UBE2A gene - case report and literature review

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Microdeletion Xq24 encompassing SLC25A43, SLC25A5, CXorf56, UBE2A, NKRF, and two non-coding RNA genes, 0.24-0.37 Mb in size, was described in five males from four unrelated families (Honda et al, 2010; Leeuw et al, 2010). Patients presented with intellectual disability, absent speech, seizures, craniofacial dysmorphism, multiple brain and inner organs anomalies.
Here we describe a 4-months-old boy with a 0.4 Mb maternally inherited Xq24deletion. He showed neonatal hypoglycaemia, low voice, swallowing difficulties, oesophageal atresia with tracheo-oesophageal fistula, aortic isthmus hypoplasia, postnatally developed microcephaly, multiple arachnoidal cysts and white matter changes, divergent strabismus, short neck, arachno- camptodactyly, vertebral anomalies, hypospadias as well as developmental delay, vision and hearing impairment. Marked dysmorphic features include: wide fontanels, hypertelorism, palpebral fissures slant up, depressed nasal bridge, small nose, long philtrum, large mouth with thin vermilion of the lip, crumpled ears. Initial diagnose severe form of Beals syndrome was ruled out by a negative FBN2 gene analysis. SNP-array analysis (Affymetrix 250k NspI) detected a 0.4 Mb Xq24 deletion which spans between base pair 118303222-118699727 (hg 18). The healthy mother was a carrier of the same deletion which originated de novo with her. She showed a complete skewed X-inactivation. A copy number variation, 1.3Mb deletion of 1p12, was detected in the boy, his mother and the maternal grandfather.
Clinical and molecular data evaluation of all six patients with deletion Xq24 suggests a new deletion syndrome different than the UBE2A deficiency syndrome. The role of the other genes involved in the deleted region remains to be further investigated.

P02.087
X-Inactivation studies in two different subtypes of Dent disease

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Background. A subset of X-linked genes escape X-inactivation (XI) and are expressed - randomly or skewed - from both X-chromosomes. We report on 2 patients affected with 2 genetically different forms of X-linked hypercalciuric nephrolithiasis (Dent disease 1 and 2) mapped to Xp11.22 and Xq26, respectively. We hypothesized that X-inactivation patterns might suggest the more likely genetic subtype. Here we discuss the clinical impact of XI results.
Case reports and results. 2 male unrelated normally developed patients, 4 and 5 y old, both with proteinuria, hypercalciuria, hypouricaemia and microhematuria with normal renal function. Dent disease, an X-linked proximal tubular dysfunction disorder, was confirmed by molecular analysis showing a mutation in the CCl5-gene in patient 1 and OCRL1 mutation patient 2. X-inactivation studies revealed a significant skewing in the mother of proband 1 and random XI in the other.
Discussion. Non-random X-inactivation is more common in heterozygous carriers of X-linked disorders and can be an important diagnostic clue in MRX-syndromes. Moreover, there may be a distinct correlation between the clustered fashion of genes which escape inactivation and the likelihood of skewing. Our observation of clearly different XI-patterns in two clinically similar but genetically different subtypes of an X-linked tubulopathy supports this relationship. Given the multitude of X gene loci of interest this may be useful for the proper timing of molecular investigations. Additional XI analyses in other females heterozygous for Dent disease are needed to prove this hypothesis.

P02.088
Desbuquois dysplasia type I and fetal hydrops due to novel mutations in the CANT1 gene

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We report on three hydropic fetuses of 17, 22 and 25 gestational weeks from three distinct families presenting with Desbuquois dysplasia type 1. All fetuses showed brachymelia and characteristic dysmorphic features. X-ray studies revealed delta-shaped extraphalangeal bones and disease-specific prominence of the lesser trochanter, varying in severity with fetal age. Early lethal manifestation of the disorder was reflected in lung hypoplasia and in early death of similarly affected siblings in cases 1 and 2. All families were German by descent. Sequence analysis of the CANT1 gene revealed two frameshift mutations, c.228_229insC and c.277_278delCT, in homozygous and compound heterozygous configuration respectively and a homozygously novel missense mutation, c.336C>A (p.D112E) located within a higly conserved region of exon 2. Haplotype analyses by high-resolution single nucleotide polymorphism array showed that the haplotype associated with c.228_229insC may be traced to a single founder in the German population.

P02.089

Yield of genetic testing in patients with familial vs. non-familial idiopathic dilated cardiomyopathy.

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Purpose: It has been estimated that 20% to 50% of idiopathic DCM cases are of familial/genetic origin, generally with autosomal dominant inheritance, but information about the efficiency of mutation screening is scarce.

Methods : We report the results of ongoing genetic and familial study of 125 index cases with idiopathic DCM (mean age at diagnosis 44±20 years, 74% males) followed-up in a single reference unit. DCM was considered “familial” when there was at least one relative with DCM. Genetic study has included the screening by a Sequenom platform of 600 previously described mutations and the sequencing of LMNA in all the patients.

Results: Familial DCM was present in 29 cases (23%). Till now we identified a potentially pathogenic mutation in 21 cases (13%): 9 of 29 in cases with familial DCM (31%), and 12 in 96 (13%) in non-familial cases (p=0.024). The majority of them were identified in MYH7 (45%) MyBPC3 (25%) and LMNA (20%). Thirteen mutations had been previously described in association with either DCM (LMNA: R190W, S573L; MYH7: I201T; TNNT2: R131W), HCM (MYH7: G716R ; MyBPC3: IVS23+1G>A, A833T, V771M and A627V) or both (MYH7: I736T, R787H, T1019N and MYH6: A1004S). Five mutations had not been previously described (LMNA:S22X, R349L; MyBPC3: P873L, E838Q and MYH7: A1128T). We found two double and one compound heterozygote. Conclusion: The probability to identify a causal mutation was significantly higher in patients with familial vs. non-familial idiopathic DCM thus, familial evaluation would be the first step to improve the mutation screening efficiency in DCM.

P02.090

Possibilities and limits in facial dismorphic syndrome in small child -disscutions on a Hallermann-Streiff syndrome case

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Introduction: Hallermann-Streiff syndrome (HSS) is a disorder of unknown origin. The early diagnosis of HSS is important for management because many complications may occur early in this syndrome.

Case report: We report N.D, a 1 year 4 month old boy, born at term, with 3010g , 49 cm , Apgar 9, the second child of a non consanquineous family. The child was admitted to our hospital for seizures, fever secondary to pharyngitis and dysmorphic facial features. Anthropometric criteria revealed short stature (height 73 cm, below 3rd percentile) and dystrophy (weight 9200 g, below 3rd percentile). The child presented with an unusually small mouth, small teeth with dentinal spots and improper alignment, small chin, small eyes, incipient cataract; small head and a prominent forehead, a small underdeveloped jaw, sparse hair, particularly that of the scalp, brows, and lashes. On the right cheek there was a scar secondary to surgical intervention for a mucous cyst done in the neonatal period. The karyotype of the child was 46, XY, 16qh+. The X-ray of the skull showed hypoplastic facial bones, large skull and frontal bossing. Clinical and radiological findings from our child meet six of seven criteria of HSS reported by Lee, Choi and Jung (dental anomalies, dischephalya, dwarfism, hypotrichosis, congenital cataract, microphthalmia).

Conclusions:

1. Hallermann-Streiff is a rare syndrome but underdiagnosed.
2. The disease is transmitted in a dominant manner but there are no specific anomalies of the karyotype.
3. Sophisticated genetic analysis is required for diagnosis of this syndrome.

P02.091

DNA-diagnostics of various forms of X-linked primary immunodeficiencies at the Russian patients

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Primary immunodeficiencies (PID) are the hereditary diseases caused by rough infringement of functions of various links of immunity. They grow out of the genetic defects leading to infringement proliferative, a differentiation and functions immune cells. By this time are known about 80 PIDs. Despite their relative rarity, they occupy an important place in structure of disease and death rate in the developed countries. PIDs are characterised by difficultly recurrent infections, a high incidence of autoimmunity, and an increased risk of malignancies.

The greatest quantity are X-linked forms of congenital immunodeficiency though there are also other types of inheritance. In laboratory of DNA-diagnostics of the Research centre for Medical Genetics of the Russian Academy of the Medical science the molecular genetic analysis of the reasons of such diseases as is carried out: X-linked hyper-IgM syndrome (CD40L gene), X-linked ag-globulinemia (BTK gene), Interleukin-2 receptor gene deficiency (IL2R gene), X-linked lymphoproliferative disease (SH2D1A gene), Wiskott-Aldrich syndrome (WASP gene) and chronic granulomatous disease (CYBB1 gene).

Presence of a certain mutation at patients with PIDs allows to show a molecular aetiology, to establish heterozygous carriers in relatives in a probands family and does possible performance of the prenatal diagnosis.

We made DNA-diagnostics for 189 probands with various forms of primary immunodeficiencies, 110 various mutations are registered and 22 prenatal diagnostics are executed.On the basis of the accumulated data (a spectrum of mutations, presence of “hotspots”) in laboratory are developed effective algorithms of the mutational analysis of various forms of PIDs.

P02.092

Whole genome sequencing enables optimized patient management

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Whole Genome Sequencing (WGS) has revolutionized genetic diagnoses, but its impact has not yet been realized in guiding immediate patient treatment. Dopa Responsive Dystonia (DRD) is a genetically heterogenous, clinically complex, movement disorder that can be misdiagnosed as cerebral palsy. Although L-DOPA is the primary treatment mode for DRD, alternate treatments are available, however, diagnosis and optimal management of the disorder requires extensive clinical investigation. A 14 year old fraternal male and female twin pair with a 9 year diagnosis of DRD was identified. Prior treatment

was exclusively L-DOPA with carbidopa. Inheritance was apparently recessive but point mutation negative for TH and GCH1. We interrogated the genomes of both affected siblings by whole genome sequencing (WGS) and array comparative genomic hybridization (aCGH) to identify causative variants. Three genes with compound heterozygous, protein altering, mutant alleles were identified. Two of these genes (ZNF515, C2orf16) have no known function whereas homozygous mutations in the third gene, sepiapterin reductase (SPR) have been previously associated with DRD. The alleles in SPR were inherited from heterozygous carrier parents. Interestingly the carrier mother has been diagnosed with fibromyalgia; a condition that can be responsive to serotonin reuptake inhibitors. Our findings conclude a specific molecular diagnosis, suggest optimizing medical management with L-DOPA and Carbidopa using 5-hydroxy tryptophan (5-HTP) supplementation in the DRD affected twins.

P02.093
Hypothyroidism and oxidative stress in Down syndrome

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Background and aims: Thyroid disorders in Down syndrome (DS) subjects have been reported to have a prevalence of 15-54%. The spectrum of thyroid abnormalities ranges from congenital hypothyroidism, primary hypothyroidism, autoimmune thyroiditis, compensated hypothyroidism. The existence of a DS-specific thyroid disorder was hypotized. Thyroid hormones are the most important factors involved in the regulation of the basal metabolic condition and are associated to the oxidative and antioxidative status. Literature's data on the oxidative status of hypothyroidism are limited and controversial. The aim of this study was to assess the measurement of serum MDA/HNE in DS patients with or without hypothyroidism, to evaluate biomarkers of oxidative stress. Methods: we enrolled a selected group of 30 children affected by DS (18 male and 12 female, mean age 6 years), 15 with hypothyroidism (8 in LT4 therapy) and 15 without. All subjects had not inflammatory/infective diseases. Serum MDA/HNE levels were measured by means of a colorimetric assay. Results: serum MDH/HNE levels were significantly higher ($p<0.05$) in hypothyroid subjects without LT4 treatment than in those euthyroid and on LT4 therapy. Conclusions: the present study evidences an increased circulating MDA/HNE levels in DS. We confirm the presence of chronic oxidative stress in children with DS. This condition can contribute to development of consequent oxidative cell damage and long-term complications. In DS with hypothyroidism, therapy with LT4 reduces oxidative stress.

P02.094
Dynamic frequency of Down syndrome in the Republic of Moldova during the years 2005-2009

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Prevention of birth of children with chromosomal abnormalities, especially with Down syndrome (DS), represents the main task of medical genetic services. Average frequency of trisomy 21 or DS is 9.5 / 10,000 births in the countries EUROCAT. Was established correlation between frequency of DS and maternal age. The risk of having children with DS is 1/1000 births for 20 years old mothers, rising to 1/35 in pre-menopausal age births.

Totally 232 cases with DS have been identified during the period 2005-2009, according to the cytogenetic laboratory data of the NCRHMG. From this number, 209 cases have been detected after the birth of children. Other 23 DS cases have been identified during the interruption of pregnancy after prenatal medical indications. The frequency of DS in the population of Republic of Moldova was 12.02 / 10 000 (1:924), registered during the years 2005-2009, which exceeds the average frequency of this pathology in countries EUROCAT and corresponds to the frequency of Down disease in Ukraine (12.4 / 10,000) and Norway (13.1 / 10,000). Maximal frequency of DS has been recorded in 2005 - 1:967 children, minimal in 2008 - 1:685 births with this chromosomal abnormality. Frequency of DS in Moldavian population could be 1:832, without applying of prenatal cytogenetic diagnostic methods. Using the cytogenetic method of prenatal diagnosis allows us to prevent the birth of children with DS and to decrease the frequency of this disease by 10%.

P02.095
Genetic polymorphisms of MTHFR (677T AND 1298C) and homocysteine metabolism as maternal risk factor for Down 's syndrome patients in North Indian population.

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Background &Aim: Down syndrome (DS) is caused by the presence of three copies of chromosome 21, in most cases due to the failure of chromosomal segregation during maternal meiosis, as there was scanty data available from India the study was designed to investigate the MTHFR C677T and A1298C polymorphisms, with homocysteine as a maternal risk factor for DS. **Materials and Methods:** Eighty mothers of individuals with confirmed full trisomy 21, and 100 control mothers of north Indian population were evaluated. Molecular analysis of MTHFR C677T and A1298C polymorphisms was performed by PCR-RFLP. **Results:** The frequency of genotypes of *MTHFR were* 677CC (82.5%), 677CT (15%) and 677TT (2.5%) in the patients with Down's syndrome, and among the 100 individuals of the control group, genotypes 677CC (91.0%), 677 CT (6.0%) and 677TT (3.0%) were found. As for polymorphism 1298C, the patients with Down's syndrome presented genotypes with frequencies 1298AA (15.0%), 1298AC (52.5%) and 1298CC (32.5%) respectively and in the control group the frequencies of genotypes were 1298AA (61.0%), 1298AC (22.0%) and 1298CC (17.0%) respectively. Homocysteine concentrations were significantly different in women with an *MTHFR* 1298CC genotype according to the groups, being higher in DS mothers than in control mothers. **Conclusion**: No correlation was observed in *MTHFR* gene polymorphism 677T in DS. However, the *MTHFR* gene polymorphism at position 1298, mainly genotype 1298CC that reduces the enzyme efficiency, was more frequent in the group of Down's Syndrome patients, suggesting its involvement in mechanisms related to chromosomal imbalances.

P02.096
Characterization of dysmorphic features in 1q21.1 microduplication syndrome: description of two patients and review of the literature

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Array-CGH screening in large patient cohorts with unexplained mental retardation, congenital anomalies or autism has recently lead to the characterisation of novel microdeletion/microduplication syndromes. The low-copy-repeats spanning the region 1q21.1 mediate nonallelic homologous recombinations that result in rearrangements of 1q21.1.

Recent data suggest that a recurrent 1,35 Mb microduplication on chromosome 1q21.1 predisposes to autism spectrum disorder, developmental delay and mental retardation. Other clinical features include macrocephaly, hypertelorism and a wide range of congenital anomalies. Variable expressivity, incomplete penetrance and non-specific phenotypic features associated with 1q21.1 microduplication complicate genetic diagnosis and counselling.

We report on two unrelated girls of two and three years old, presenting with developmental delay and similar facial dysmorphism such as hypertelorism, flat nasal bridge, short nose with anteverted nostrils, prominent philtrum and thin upper lip. Head circonferences were in normal ranges. At the age of three, one of them developed behavioral disorder with hallucinations and aggressivity.

Array-CGH detected an identical 2.5Mb microduplication on 1q21.1, encompassing the minimal critical region of 1.35Mb. Parental origin is undetermined (paternal DNA was not available for analysis).

These two additional patients confirm that identification of 1q21.1 microduplication by array-CGH should be considered as a predisposition factor for developmental delay and behavioral disorders. We refine the 1q21.1 microduplication syndrome by suggesting a distinctive common phenotype, comparing facial dysmorphic features of our patients with previously reported cases.

P02.097
Spondylocostal dysostosis associated with duodenal diaphragm: a case report.

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We report a case of a 2-year-old boys, the only son of healthy non consanguineous parents. He presented skeletal abnormalities, short stature with short trunk in proportion to height, and dimorphisms (facial asymmetry, strabismus, micrognathia, low-set ears, two preauricular tags right, short neck). The radiological picture shows multiple abnormalities of the spine (hemivertebrae, absence of vertebrae, vertebral hypoplasia), fusion of ribs (5-6-7 right) and malformation (6th left). These skeletal abnormalities are diagnostic of the spondylocostal dysostosis, a rare condition of variable severity. The disease is inherited as an autosomal recessive or autosomal dominant and four genes known are associated with autosomal recessive spondylocostal dysostosis: DLL3, MESP2, LFNG and HES7. The condition is characterized by multiple segmentation defects of the vertebrae (fusion of vertebrae, hemivertebrae), presence of malalignment of the ribs with variable points of intercostal fusion, and a reduction in rib number. Deformity of the chest with reduction of size of the thorax, short neck, scoliosis, inguinal hernia are frequent complications of this condition. Spina bifida, meningocele, renal abnormalities, hypospadias, congenital heart disease, anomalous pulmonary venous return have been reported, but are not common. The our proband was affected by duodenal diaphragm, characterized by the presence of a mucosal diaphragmatic membrane with an intact muscle wall. He presented bilious vomiting ,feeding intolerance and failure to thrive in neonatal period. At one years old, he underwent surgical correction. We report the first case, in absence of other data from the international literature, of spondylocostal dysostosis associated with duodenal diaphragm.

P02.098
The arthrochalasis type of Ehlers-Danlos syndrome: phenotype at different ages

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Ehlers-Danlos syndrome (EDS) is a clinically and genetically heterogeneous group of inherited connective-tissue disorders characterized by hypermobility, tissue fragility and skin abnormalities. Six subtypes have been characterized based on clinical features and molecular genetic abnormalities. The arthrochalasis type EDS (former type VIIa and VIIb) is characterized by bilateral congenital dislocation of the hips, severe generalized joint hypermobility with dislocations, muscular hypotonia and distinct dysmorphic features. Early diagnosis of arthrochalasis type EDS is of importance because of consequences for mobility in later life. However, the differential diagnosis may be difficult because of overlap with other hypermobility syndromes. We describe seven patients at different ages. Timing of diagnosis varied from antenatally to adult age. Genetic counselling is possible at the adult age.

Diagnosis was confirmed in all patients by biochemical studies or mutation analysis showing characteristic mutations in COL1A1 and COL1A2, resulting in skipping of exon 6, leading to the production of abnormal procollagen and defective collagen synthesis.

For physicians treating patients with EDS type VII achieving mobility for the patient is the greatest challenge. The prognosis for achieving independent walking may be poor due to recurrent dislocations of nearly all joints in severe cases. We stress the importance of a diagnosis at various ages.

P02.099**
Musculo-contractural Ehlers-Danlos syndrome (EDS type VIB): a new EDS-subtype caused by a defect in proteoglycan biosynthesis

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Musculo-contractural Ehlers-Danlos syndrome (MC-EDS) is a recently described autosomal recessive form of EDS characterized by craniofacial abnormalities, congenital joint contractures, especially of fingers and toes, talipes equinovarus, tapering fingers and excessive palmar wrinkling, severe progressive muscle hypotonia and multi-systemic tissue fragility with ophtalmological, gastro-intestinal and genito-urinary manifestations, in addition to skin fragility, wound healing problems, bleeding diathesis and joint hyperlaxity. The condition is caused by homozygous mutations in CHST14, encoding dermatan-4-sulfotransferase 1 (D4ST-1). The clinical phenotype of MC-EDS strongly overlaps with that of adducted thumb-clubfoot syndrome (ATCS), and with a Japanese EDS-variant, “EDS Kosho type”, two conditions that were independently shown to be caused by CHST14 mutations. Detailed phenotypic analysis of the 20 patients reported with CHST14 mutations strongly suggests that these conditions form a single entity with multi-systemic, but age-dependent and variable phenotypic features, of which joint contractures and generalized muscle weakness are distinguishing features, hence the name “musculo-contractural EDS”.

Hitherto reported CHST14 mutations comprise six missense, two frameshift and one nonsense mutation, some of which were shown to result in loss of function of D4ST-1. This is a key enzyme in the biosynthesis of dermatan sulfate (DS), catalyzing 4-O-sulfation of N-acetyl-galactosamine. Loss of D4ST-1 activity results in the replacement of DS by chondroitin sulfate. The broad array of physiological events in which DS plays a role, including the correct assembly of collagen fibrils mediated by decorin, a DS proteoglycan, explains the multisystemic manifestations of this condition.

P02.100
Clinical and molecular characterization of a patient with Osteogenesis Imperfecta / Ehlers-Danlos phenotype: new mutation in COL1A1

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Introduction: Ehlers-Danlos syndrome (EDS) is a connective tissue disorder characterized by skin hyperextensibility, abnormal wound healing and joint hypermobility. Clinical and genetic heterogeneity is described. Classification of patients in the several subtypes is sometimes difficult. We report on a patient with Osteogenesis Imperfecta / Ehlers-Danlos phenotype (OI/ED) and COL1A1 gene mutation.

Clinical Report: The patient, a 6 years old boy, was referred at 17 months of age with clinical diagnosis of Osteogenesis imperfecta. Motor retardation, fractures, osteopenia, blue sclerae, macrocephaly, short stature and dysmorphic features. Physical examination revealed fine hyperextensible skin, abnormal healing, easy bruising, hyperextensible joints, lumbar kyphoscoliosis, pectus excavatum, long fingers and toes and inguinal herniae. Clinically suggestive of Ehlers Danlos syndrome. We started treatment with byphosphonates due to bone fragility. Biochemical collagen analysis in cultured fibroblasts was performed with identification of an extra band migrating above the type I collagen chain. Molecular analysis of COL1A1 gene revealed a de novo heterozygous mutation in exon 8 c.607G>T (p.Gly203Cys, Gly25Cys). These results support clinical diagnosis of combined OI / ED phenotype.

Discussion: A new mutation associated to OI / ED phenotype is described. This report extends the range of phenotypes caused by COL1A1 gene mutations. Consider treatment with byphosphonates in patient with Ehlers Danlos syndrome and bone fragility.

P02.101
Intragenic EHMT1 mutations in French patients with Kleefstra Syndrome

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The Kleefstra syndrome is one of the first and most common clinically recognisable telomeric syndrome characterized by intellectual disability, chidhood hypotonia and distinctive facial features. In addition, heart and urogenital defects, epilepsy and behavioural problems are also observed. The syndrome is either caused by a 9q34.3 submicroscopic deletion (75 %) or by intragenic euchromatin histone methyl transferase 1 (EHMT1) mutations leading to haploinsufficiency of the gene.

We evaluated 42 patients referred by clinical geneticists presenting with the core phenotype of the syndrome but no deletion identified by routine subtelomere or whole genome chromosome testing. The molecular analyses included a screen for small intragenic EHMT1 deletions by MLPA and direct sequencing of the 27 exons for point mutations identification.

In total, 9 intragenic mutations or deletions were found that are highly likely pathogenic. 4 small deletions were identified by MLPA. 5 mutations predicted to result in nonsense mediated mRNA decay were found by sequencing: 1 previously described nonsense change (p.Arg620X) and 4 novel frameshift mutations (p.Glu342fs, p.Glu187fs, p.Val1026fs, c.2018_2018 +3 del 4). De novo occurrence was confirmed when DNA from both parents were available. As previously described, mutations were spread all over the gene.

Frequently associated features reported in the Kleefstra syndrome such as obesity, congenital heart defect or epilepsy do not seem to be as frequent in our patients suggesting that genes, apart from EHMT1, included in larger deletions may be implicated in the phenotype.

P02.102
Copy number variations in patients with electrical status epilepticus in sleep

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Background: Electrical Status Epilepticus in Sleep (ESES) refers to an electroencephalographic pattern of continuous, subclinical sleep induced spikes-and-waves. ESES related epilepsy syndromes share an association of infrequent seizures, deficits in language or global cognitive functioning and behavioural problems. The aetiology is often unknown, but genetic risk factors have been implicated.

Aim of the study: To detect copy number variations (CNVs) in patients with ESES syndrome in order to identify possible underlying genetic risk factors.

Methods: We included a consecutive series of patients with ESES between January 2000 en August 2009, at the Paediatric Neurology outpatient Clinic of the University Medical Centre Utrecht. CNVs were identified by array comparative genomic hybridization. To gain more insight into the pathogenic contribution of the identified CNVs, we reviewed the literature, searched in databases for similar CNVs in patients and studied the function of the genes located within the CNVs. We compared the clinical characteristics of patients with CNVs and patients without CNVs

Results: We detected seven CNVs in four of the thirteen patients with ESES, which consisted of six novel gains and one loss, the recurrent 15q13.3 microdeletion. The copy number variant genes in two of these patients belonged to the cholinergic pathway. There were no differences in clinical characteristics between patients with CNVs and patients without CNVs.

Conclusion: Our data on CNVs in patients with ESES indicate that specific chromosomal loci and genes may be involved in the aetiology of ESES. In particular, our findings point towards putative alterations in the cholinergic pathway.

P02.103
Mutations in ENPP1 associated with a new phenotype including hypophosphatemic rickets, skin abnormalities and hearing loss

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Generalized arterial calcification of infancy (GACI) is an autosomal recessive disorder due to mutations in ENPP1 in most cases, and characterized by diffuse vascular and periarticular calcifications, frequently leading to death in early infancy. A few patients present with a milder phenotype and hypophosphatemic rickets. Pseudoxanthoma elasticum (PXE) is another autosomal recessive multisystem disorder characterized by ectopic mineralization and fragmentation of elastic fibers of connective tissue including skin, arteries, and the eye. Classic PXE results from mutations in the ABCC6 gene.

We report on two unrelated children with an unusual phenotypic overlap of GACI and PXE. They both presented with neonatal articular and vascular calcifications, with favourable evolution. Later in infancy, both patients developed abnormal mineralization in a number of organs, including ear ossicles, kidney, and eyes, associated with hypophosphatemic rickets. Angiomatous cutaneous lesions were noted in both patients. The older patient presented also with PXE skin lesions, confirmed by histology. In both patients presence of ENPP1 mutations, and absence of ABCC6 mutations was demonstrated.

This particular phenotype, overlapping between PXE and GACI appears to be associated with a better prognosis than the classical GACI with respect to vascular calcifications.

Since ABCC6 was recently discussed as the causative gene in a case of GACI without mutations in ENPP1, we demonstrate here that, conversely, ENPP1 may be involved in some cases of PXE in the absence of ABCC6 mutations. We conclude that ENPP1 and ABCC6 are probably involved in the same molecular pathway.

P02.104
Familial study of R217C mutation in Exon 7 of CHRNG gene in a case of multiple Pterygia Syndrome

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BACKGROUND: Escobar or multiple pterygia syndrome (OMIM 265000), a form of arthrogryposis multiplex congenital, is an autosomal recessive condition characterized by excessive webbing (pterygia), congenital contractures (arthrogryposis), and scoliosis. Congenital contractures are common and may be caused by reduced fetal movements due to myasthenia gravis. Hereditary causes of congenital myasthenic syndrome have also been identified, mostly mutations in subunits of nicotinergic acetylcholine receptor (AChR). The AChR is composed of five subunits; two alpha, one beta and one delta subunit are invariably present. The gamma subunit (CHRNG gene) is present before the 33rd wk of gestation in humans but is replaced by a subunit in the late fetal and perinatal period, thereby forming the adult AChR.

MATERIALS AND METHODS: Proband case was a four year old male. On clinical examination, the patient had all the characteristic features of multiple pterygium syndrome. He was of short stature, with flexion deformities of the wrists and hands and webbing across all flexion creases. Bilateral foot deformities were present with a right-sided rockerbottom foot deformity and a residual club foot on the left.

RESULTS AND CONCLUSIONS: Genetic study of CHRNG gene was performed, patient was carrier of R217C mutation in exon 7 (c.715C>T) in homozygosis. Study of other members of family detected 35 heterozygotic carriers and till three different familial pedigrees affected. CHRNG gene mutations have been identified in families with Escobar syndrome and showed that the trait is a congenital dysmorphology caused by the transient inactivation of the neuromuscular end plate.

P02.105
Submicroscopic 7q36.1-36.3 deletion causes multiple congenital eye anormalies, associated with short stature in a boy with normal development

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The eye is a very complex structure that originates from constituents derived from a number of sources, such as the neuro- and surface ectoderm and the periocular mesenchyme, which receives contributions from both the neural crest and mesoderm lineages. Proper eye development requires many interacting proteins, such as PAX6 and SHH, which orchestrate a series of sequential inductions. Due to the complexity of eye development, it is not surprising that congenital eye abnormalities occur, particularly in multiple malformation syndromes.

We report the case of patient LG, a 14 year old boy, referred to the Medical Genetics clinic due to multiple congenital eye abnormalities (unilateral microphthalmia, bilateral microcornea, severe myopia, nystagmus, dysplasia of the optic disc with coloboma-like areas, vitreo-retinal degeneration), proportionate short stature, absence of the two maxillary central incisors and congenital unilateral vertical astragalus. Brain MRI scan, heart, abdominal and reno-pelvic ultrasound were normal, evidencing the absence of additional malformations. Furthermore, he has a normal psychomotor development.

The cytogenetic investigation detected a de novo submicroscopic deletion in terminal region of the long arm of chromosome 7 [46,XY,del(7)(q36.1-q36.3). ish 7qsubtel(VY JyRM2000X2). arr 7q36.2q36.3 (153206357-156133135)X1 dn). This region encompasses 10 genes reported in the OMIM morbid map, including SHH and MYP4, both implicated in eye development and function.

This report indicates a contiguous gene disorder, causing multiple congenital anomalies, particularly affecting the eye development, in the absence of developmental delay.

P02.106
A novel mutation in the APC gene in a patient with attenuated colonic polyposis and severe extra-colonic phenotype

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We describe a woman with no relevant clinical history until age 70, when she underwent right hemicolectomy for multiple colonic polyps. Pathology examination revealed 40 adenomas, one of which, showing cancer foci, tested negative for microsatellite instability. At age 76 the patient developed an ampullar adenoma and at age 77 she underwent surgery for lung adenocarcinoma (she is non-smoker with no other lung cancer risk factors). Family history was negative for polyposis but her father and brother died at a young age for stroke, while her niece died at age 40 for medulloblastoma. Search for germline mutations in polyposis genes detected the heterozygous mutation c.6156delG (p.L2052fs) in APC, whereas MYH was wild-type. The c.6156delG mutation was found in both duodenal adenoma and lung adenocarcinoma at the heterozygous state, excluding the loss of the wild-type allele in tumor tissue.

To our knowledge, the c.6156delG mutation has not been reported before. Its location at the 3' terminus of the gene may explain the attenuated colonic phenotype in this patient, consistently with the reported genotype-phenotype correlations, but such extracolonic manifestations were unexpected. However, although molecular analysis on tumor tissue failed to find LOH, several factors suggest that the APC mutation may be the cause of the neoplasms arisen in the family: i) 50% of patients with classic FAP develop ampullar adenomas; ii) adenocarcinoma of the lung has been reported in three patients with FAP; iii) APC mutations can cause Turcot syndrome, featured by the association of colonic polyposis with the rare adult-onset medulloblastoma.

P02.107
Floating-Harbor Syndrome: report on a case in a mother and daughter, further evidence of autosomal dominant inheritance

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Floating-Harbor Syndrome (FHS) is a rare disorder characterized by the triad of proportionate short stature with delayed bone age, characteristic facial appearance and delayed speech development. The genetic basis of the syndrome is still unclear, most cases being sporadic. In the rare familial cases, three reports with either mother-daughter or mother-son transmission, fulfilling the diagnosis criteria of FHS, suggest an autosomal dominant inheritance pattern. This mode of transmission is also suggested by advanced paternal age in almost all sporadic cases. On the other hand, autosomal recessive inheritance has also been suggested in three pedigrees where the phenotype is less convincing. In two other reports, autosomal recessive inheritance seemed possible because of consanguinity although germinal mosaicism for an autosomal dominant gene mutation could not be excluded. We report on the fourth case in a mother and daughter, review the familial cases of the literature and suggest autosomal dominant inheritance for this syndrome.

P02.108
Search for a gene responsible for Floating-Harbor syndrome

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Floating-Harbor syndrome (FHS) is characterised by characteristic facial dysmorphism, short stature with delayed bone age and expressive language delay. To date, the gene(s) responsible for FHS are unknown and the diagnosis is only made on the basis of the clinical phenotype. The majority of cases appeared to be sporadic but rare cases following autosomal dominant inheritance have been reported. We identified a 4.7 Mb *de novo* 12q14.3-q21.1 microdeletion in a patient with FHS and mental retardation. Pangenomic 244K array-CGH performed a series of twelve patients with FHS failed in identifying overlapping deletions. We hypothesized that FHS is caused by haploinsufficiency of one of the 19 genes or predictions comprised in the deletion found in our index patient. Since none of them appears as good candidate genes by their function, high-throughput sequencing approach of the region of interest was used in eight FHS patients. This method is based on the production of thousands or millions of sequences at once during the sequencing process. The analysis only revealed a nonsense variant in the exon 2 on the *TMEM19* gene in one patient, but segregation analysis showed that the mutation was inherited from his unaffected mother. This approach failed in identifying the gene responsible for FHS, and it can be explained by 3 reasons: i) our index patient could be a phenocopy of FHS; ii) the disease can be clinically heterogeneous since the diagnosis only relies on clinical features, iii) high genetic heterogeneity of the disease.

P02.109
A family with frontometaphyseal dysplasia in three generations

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Frontometaphyseal dysplasia (FMD, OMIM 305620) is a rare X-linked craniofacial genetic syndrome first described by Gorlin and Cohen in 1969. It is characterized by supraorbital hyperostosis, hypertelorism,

conductive hearing loss, downslanting palpebral fissures, anodontia or oligodontia, as well as generalized skeletal dysplasia which manifests with thickening of the calvarium, agenesis of the frontal, ethmoidal and sphenoidal sinuses together with bowing and undermodeling of the diaphyses and metaphyses of the tubular bones. FMD is caused by mutations in the FLNA gene which encodes the cytoskeletal protein filamin A that binds actin fibrils and participates in the modulation of the cytoskeleton. We describe here a Greek family with five FMD cases in three generations. Three brothers had typical severe manifestations of the syndrome, while their mother and maternal grandmother had less prominent signs. Genomic DNA was extracted from all patients, as well as from the normal father of the three male patients. All 48 exons of the FLNA gene including exon-intron boundaries were sequenced in one male proband and a single mutation (D253E) was revealed. The same mutation was found in all affected members of the family, both in hemizygous males and in heterozygous females.

P02.110
High prevalence of ectopic calcification in the Azores - Gitelman Syndrome as the cause for secondary Chondrocalcinosis

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Gitelman syndrome (GS) is an autosomal recessive inherited renal disorder, characterized by defective tubular reabsorption of sodium, potassium and magnesium, due to loss of function mutations of the *SLC12A3* gene. This syndrome leads to hypomagnesemia, hypokalaemia, mild hypotension, mild metabolic alkalosis and hyperreninemic hyperaldosteronism. It is thought that hypomagnesemia is the reason for the recurrent association of GS with Chondrocalcinosis (CC).

A 59 years-old Azorean patient with CC, hypomagnesemia and hypokalemia, was identified in the Rheumatic Diseases Clinic, Hospital de Santo Espírito, Azores, Portugal. The clinical diagnosis of GS was performed and, after informed consent, genomic DNA was extracted from peripheral blood cells. *SLC12A3* direct sequencing revealed three variants: a missense substitution in heterozygous state (c.296A-G, His99Arg), a synonymous heterozygous substitution (c.366A-G, Ala122Ala) and a homozygous missense substitution in exon 15 (Ser615Leu). Mutation in exon 15 has been previously described as a genetic cause for GS.

In geographic regions, such as the Terceira Island (Azores, Portugal), in which CC and enthesopathies are considered to be extremely prevalent, it is crucial to determine its genetic causes and to establish the differential diagnosis. The genetic cause for the secondary CC in this GS patient was identified. Further studies in family members of this patient will be needed in order to evaluate the segregation of hypomagnesemia and CC.

P02.111
Deletions of the GLI3 gene can be the frequent cause of Greig cephalopolysyndactyly and preaxial polydactyly type IV

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Greig cephalopolysyndactyly syndrome (GCPS) and isolated preaxial polydactyly type IV (PPIV) are rare autosomal dominant disorders, both caused by mutations in the GLI3 gene. GCPS is predominantly characterised by craniofacial anomalies (macrocephaly, high forehead with frontal bossing, hypertelorism), and limb malformations such as preaxial polydactyly type IV, syndactyly, and postaxial polydactyly type A or B. Other congenital abnormalities are less frequent. The syndrome exhibits high inter-individual and intra-familial clinical variability. Mutations in the GLI3 transcription factor gene can also cause Pallister-Hall syndrome, and isolated postaxial polydactyly type A and B. In this study we screened 11 probands (8 familial and 3 sporadic) with the clinical diagnosis of GCPS/PPIV by gene sequencing and found 8 novel and 1 previously reported heterozygous point mutations. Subsequently we performed MLPA (P179 MRC Holland Kit) to screen for intragenic copy number changes and demonstrated heterozygous deletions in the 2 remaining cases (18%). Although based on a small sample, our study shows that intragenic GLI3 deletions may account for a considerable proportion of GCPS/PPIV causative mutations. Therefore, we propose that MLPA or qPCR should be included into routine molecular diagnostic of the GLI3 gene, especially if the sequence analysis detects no pathogenic mutation.

P02.112
Goldenhar Syndome - report of three cases

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Goldenhar Syndrome (oculo-auriculo-vertebral dysplasia), is a rare congenital complex of abnormalities with various models of expressivity.

Matherial and methods: We present three male infants with Goldenhar syndrome from Romania. Results: All cases were diagnosed in their newborn period. There was no family history of facial syndromes or ocular condition. The children have normal anthropometric parameters (head circumference, birth length and weight) and abnormalities characteristic for the syndrome (consistent features of epibulbar dermiods, pre-auricular tags, and vertebral anomalies). Investigation and complex interdisciplinary consultations highlight the similar and different abnormalities present in the three cases. The severity of the syndrome is different for each case and consequently the management, evolution and prognosis will be different. Conclusions: Due to the fact that manifestations of Goldenhar syndrome can be similar to other diseases, to confirm the syndrome, genetic testing is a valuable tool to establish the diagnosis and allow appropriate genetic counseling.

P02.113**
Looking at Gorlin syndrome from the perspective of the cilia of hedgehog

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Gorlin syndrome (GS), also known as basal cell nevus syndrome, is an autosomal dominant disorder characterized by multi-organ developmental abnormalities, and neoplasms. In up to 85% of cases of GS, a mutation can be identified in the patched homolog 1 gene (*PTCH1*). *PTCH1* gene is a member of Sonic Hedgehog (SHH), an important signaling pathway of the cilia. PTCH1 is the SHH receptor and represses the SHH signaling through inhibition of Smoothend (Smo). Mutations in *PTCH1* render it unable to repress Smo which enters the primary cilia resulting in permanent activation of SHH signaling. So far, attempts to make genotype-phenotype associations in GS have not been successful. However, no one has looked at GS from the pathogenic perspective of a cilia abnormality. We provide evidence supported by research reports that the apparently unrelated phenotypic features of GS have a ciliary dysfunction as their common underlying mechanism. Support for this hypothesis includes firstly clinical overlap (cysts, hydrocephalus, medulloblastoma, skeletal abnormalities) between GS and other ciliopathies. Secondly, the important role of cilia in the development of the involved structures in GS (i.e. brain, skin, skeleton) and their malignant counterparts (basalioma, medulloblastoma). Furthermore, mutations in *SUFU*, a ciliary protein and a negative SHH regulator, have been found in sporadic medulloblastoma, as well as in germline in a family with GS. Also *SUFU* +/- mice have features of GS. The perspective of GS being a ciliopathy can be clinically relevant for treatment with drugs targeted to downstream components of the primary cilia.

P02.114
Problems of differentiation between Huntington and Hallervorden-Spatz disease

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Huntington disease (HD) is an autosomal dominant progressive neurodegenerative disorder with a distinct phenotype characterized by chorea, dystonia, incoordination, cognitive decline, and behavioral difficulties. In the classic form age of onset is between 30 and 40 years. Hallervorden-Spatz disease is an autosomal recessive disorder characterized by extrapyramidal movements, such as parkinsonism

and dystonia. In the classic form patients present within the first decade of life with rapidly progressing disease and loss of ambulation approximately 15 years later. In classic forms differentiation of this disorders is not difficult for physicians. But there is juvenile-onset Huntington disease (10% of all HD cases), typically defined as onset before age 20 years and the atypical form of Hallervorden-Spatz disease, when patients have onset in the second decade with slow progression and maintain independent ambulation after 15 years. In our practice there were two cases wrong diagnosis "Hallervorden-Spatz disease" when patient suffered from Huntington disease. Therefore we decided to draw attention of physicians for this problem. These disorders have overall symptoms such as dystonia, choreatic athetosis, dementia, ataxia, rigidity dysarthria, bradykinesia and only patient suffered from Hallervorden-Spatz disease have deformation of foot, primary pigmentary degeneration of retina, blepharospasm, tremor, spasticity, diphthongia. The main feature of Hallervorden-Spatz disease is the 'eye of the tiger' sign on brain MRI. The main confirmation of Huntington disease is expansion of repeat number more than 37 in IT15 gene. The main confirmation of Hallervorden-Spatz disease is presence mutations in the PANK2 gene.

P02.115
Hartsfield syndrome and Xq24 microduplication

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The combination of holoprosencephaly and ectrodactyly was first described by Hartsfield et al in 1984. Fifteen cases have been reported to date and the condition is likely to represent a single genetic entity (Hartsfield syndrome). The genetic defect is yet to be unraveled. Based on the observation that male patients have preferentially been affected, X-linked recessive mode of transmission has been suggested for this condition. Yet, no candidate genes have been suggested on the X chromosome. We report a patient with a full-brown Hartsfield syndrome phenotype who had a microduplication at Xq24 that involved at least 5 genes: A Japanese boy had bilateral ectrodactyly of the hands, midline cleft lip with aplasia of premaxillary segment, cleft palate, a low nasal bridge and micropenis with undescended testes. A CT scan of the brain revealed lobar-type holoproencephary. Cleft lip and palate was repaired a age 2 years and ectrodactyly was repaired at age 3. He had multiple episodes of hyper natremia with serum sodium level as high as 194 mEq/l. His development was mildly delayed. His G-banded karyotype was normal. Array comparative genomic hybridization using the Whole Human Genome CGH array (Agilent) revealed a duplication of 0.3 Mb, on Xq24. The duplication has never been recognzied as benign copy number variation in various CNV databases. The duplication was derived from the unaffected mother who had random X-inactivation pattern. The fact that the X-inactivation pattern was not incompatible with the notion that the duplication could be pathogenic.

P02.116
A HPE-like phenotype and heterotaxy associated with a 1,3 Mb deletion encompassing the *GLI2* gene at 2q14.2

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Loss-of-function mutations of *GLI2* are associated with holoprosencephaly (HPE)-features, including abnormal pituitary gland formation and/or function, craniofacial abnormalities, branchial arch anomalies and polydactyly. We report a patient carrying a 1,3 Mb submicroscopic deletion in 2q14.2, encompassing the *GLI2* gene. She presented with a HPE-like phenotype (bilateral cleft lip and palate and abnormal pituitary gland formation with panhypopituitarism),heterotaxy and normal psychomotor development. Submicroscopic deletions encompassing *GLI2* have not been reported so far. Large microscopically visible interstitial deletions spanning 2q14.2 are rare and have been reported sporadically in patients with multiple congenital anomalies and mental retardation. The clinical and molecular findings in our proband and her family are presented. Additionally, we review the features of previously reported index patients with a *GLI2* aberration (17 mutations and 7 large deletions spanning 2q14.2). With the report of this *GLI2* deletion we confirm that haploinsufficiency of *GLI2* is associated with HPE-like features. Comparable to what has been described in families with *GLI2* mutations, we observed an incomplete penetrance of the deletion in our family, illustrating the multifactorial etiology of HPE and HPE-like features. The deletion contains five other genes, including the *Epb4.1l5* gene. Based on observations in *Epb4.1l5* mutant mice embryo's, we argue that haploinsufficiency of *Epb4.1l5* might be the cause of the heterotaxy in our proband. A pathogenic effect of the *GLI2* deletion cannot be excluded. Finally, we conclude that, while other HPE-genes have been excluded, *GLI2* is still a candidate gene for the Holoprosencephaly-polydactyly syndrome, also known as Pseudotrisomy 13.

P02.117
Alobar holoprosencephaly in two sibs: deletion of chromosome 17

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Most of the reported cases on HPE are sporadic, representing nonrandom chromosomal aberrations and environmental agents, however, familial cases are described in some mendelian genetic syndromes. HPE recurring in sibs has been also reported as part of the clinical spectrum of some well recognized autosomal or x-linked recessive syndromes such as the pseudotrissomy 13 syndrome, Genoa syndrome, Lambotte syndrome, Smith-Lemli-Opitz syndrome, among other less frequent. Mutation in genes such as SHH, GLI2, PTCH, TGIF, and SIX3 have been recently reported in patients with different types of HPE, in parents with minor anomalies, and in normal carrier parents with an autosomal dominant patter of inheritance. In the present report we describe two affected brothers with alobar HPE and one of them represent a case of recurrence in sibs with normal parents. Mutation screening for the genes SHH, GLI2, PTCH, TGIF, and SIX3 was carried out. No evidence of mutation was found. Then, aCGH analysis was carried out and a chromosome 17 deletion affecting the p12 band between 14052279 and 15102307 bp was found. The deficiency of this fragment has been associated to the hereditary neuropathy with liability to pressure palsies (HNPP). It is possible that the phenotype is associated to this chromosomal alteration. Genetics and clinical aspects involving the present family and recurrence risks involved are discussed.

P02.118
Duplication of TBX5 and TBX3 associated with Holt-Oram-Syndrome plus supernumerary mammary glands

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Loss of function mutations in TBX5 are associated with Holt-Oram-Syndrome (HOS), an autosomal dominant disease characterized by radial limb defects and heart defects. Loss of function mutations in TBX3 result in Ulna-Mammary-Syndrome (UMS), an autosomal dominant disorder associated with ulnar limb defects, hypoplasia of breasts, and dental abnormalities. Here we report on a German family in which a duplication of the two adjacent genes TBX5 and TBX3 was identified. Affected members in five generations suffered from mild hand anomalies, heart defects, and supernumerary mammary glands. The phenotype except accessory mammary glands resembled HOS. Sequencing of all TBX5 exons was performed with inconspicuous results. Using a SNP oligonucleotide array, duplication at 12p24.21 was detected extending 210 kb and encompassing exclusively the TBX5 and TBX3 genes. To our knowledge duplication of both TBX5 and TBX3 genes have never been described before. Previously published studies have shown that TBX5 gain of function mutations are associated with HOS with mild skeletal involvement. Based on this knowledge the mild hand malformations in the family described here are likely caused by duplication of the TBX5 gene. Taking into account that TBX3 loss of function mutations result in hyploplastic breasts development, supernumerary mammary glands observed

in our cases are likely caused by duplication of the TBX3 gene. We assume that duplication of TBX5 is associated with a HOS with milder skeletal involvement and duplication of TBX3 apparently leads to supernumerary mammary glands.

P02.119
Biallelic absence of TRMP1 may explain the retinopathy in patients with homozygous 15q13.3 microdeletions

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The 15q13.3 heterozygous microdeletion is a new frequent recurrent microdeletion with high clinical variability and incomplete penetrance, with an average size of 1.5 Mb. Rare patients have been described in the literature with a homozygous microdeletion inherited from both parents. We describe 3 patients from a French cohort, aged 8 months to 6 years, including one sibship, with a 1.5 Mb homozygous microdeletion. The parents had mental retardation in one family only. They all had congenital blindness with a peculiar rod-cone dystrophy confirmed by electroretinography. The two oldest patients had convulsivant encephalopathy, major hypotonia, and one of them had choreoathetosis movements. Contrary to the heterozygous microdeletion, the homozygous 15q13.3 microdeletion displays an identifiable clinical phenotype. The neurological symptoms might be explained by homozygous deletion of CHRNA7. Taking into account another patient from the literature with a homozygous deletion not including TRPM1 in one allele and absent eye manifestations, we conclude that homozygous deletions of TRPM1 might be responsible of the retinopathy. This data is supported by the finding that homozygous mutations lead to congenital stationary night blindness. In conclusion, the association of retinopathy and convulsive encephalopathy should lead to the search of a 15q13.3 homozygous microdeletion.

P02.120
Interstitial duplication of HUWE1 supports the role of this gene in X-linked mental retardation.

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Ubiqitin ligases have been implicated in various neurogenetic disorders, most notably Angelman Syndrome (UBE3A). The HUWE1 ubiquitin E ligase gene is an X-linked gene involved in the ubiquitination of proteins in the nervous system. It functions upstream of the N-Myc-DLL3-Notch pathway and plays a crucial role in regulating the balance between proliferation and differentiation of neural cells. Mutations in HUWE1 have been described in a family with Turner-type mental retardation associated with macrocephaly (OMIM #30706) and 2 families with nonsyndromic XLMR. As well, 6 unrelated families have been reported with various duplications of the Xp11.2 region, encompassing both HSD17B10 and HUWE1. We present a male child with severe MR, partial complex seizures, and moderate to profound sensorineural hearing impairment. He was nondysmorphic as a child, but at 15 years, had poor eye contact, with long narrow face and anterior maxillary overbite. MRI showed multiple hyperintensity foci on the T2 flare images with a large area involving the right temporal lobe. Array CGH demonstrated a microduplication involving the HUWE1 gene within cytogenetic band Xp 11.22. This represents a partial duplication of the gene with minimum size of 55 KB from exons 37 to 84 and maximum size of 99 KB including exons 24 to 84. His mother and 2 of his maternal half-sisters have the same duplication and mild learning disabilities. This case represents the first description of an interstitial duplication of HUWE1 and supports the implication of HUWE1 alterations in abnormal brain development and functioning.

P02.121
Comparison of prescreening methods and their effectiveness in diagnostic of hypertrophic cardiomyopathy

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Familial hypertrophic cardiomyopathy (HCM) is characterized by progressive hypertrophy of left (sometimes also right) ventricle, diastolic dysfunction, heart failure and sudden death. HCM is heterogenic disease, penetrance is incomplete with variant expressivity. Heredity is autosomal dominant with frequency 1/500. Genetic heterogeneity is caused by mutations in approximately 12 genes that encode sarcomeric complex and others.

Mutations in 3 genes: *MYH7* (gene encodes heavy chain of β-myosin), *TNNT2* (gene encodes troponin T), and *MyBPC3* (gene encodes myosin binding protein C) cause 2/3 of all cases of HCM.

Because of the high frequency of disease and heterogeneity of HCM mutation analysis is extremely laborious and time-consuming. Neither the clinical phenotype is not guideline to genetic analysis, because there are no specific symptoms that would correlate with mutations in genes. The only option is to follow the frequency of mutations in the genes: MYH7, MYBPC3, TNNT2, TPM1, MYL2, MYL3, TNNI3 and others. We compare and discusse the sensitivity and specificity of SSCP, DGGE and heteroduplex analysis and we propose testing of patients with HCM. The results put further emphasis on the need for cooperation with cardiologist, clinical genetics and molecular genetics from diagnosis to interpretation the results of molecular genetic testing.

P02.122
Genetic diversity of Myosin-Binding Protein C (MyBPC3) gene in Russian patients with hypertrophic cardiomyopathy (HCM)

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Background: Primary HCM is an inherited cardiac disorder characterized by clinical and genetic heterogeneity. Mutations in the *MyBPC3* gene account for 20% of HCM cases and are usually associated with a favourable prognosis. Despite the good survival of *MyBPC3* mutation carriers, they may also be at risk of sudden cardiac death (SCD).

Methods: A panel of 30 patients with primary HCM was formed. We screened coding and adjacent intronic areas of the *MyBPC3* gene by direct sequencing in 4 patients. Patients had a medical examination, personal and family history was collected, physical examination, standard ECG, 24-hr HM and Echo-CG.

Results: Two known mutations (S217G, Q1233ter), 2 known HCM-associated polymorphisms (S236G, R326Q) and 2 new variants (c.2067+38 A>T, V449E) were found. One patient carried heterozygous variants R326Q, Q1233ter and c.2067+38 A>T, had early manifestation, fast progression and a positive SCD family history. Compound heterozygosity of Q1233ter and R326Q variants was previously described as associated with HCM but without SCD events. A patient with a mild form of HCM had a heterozygous S217G mutation shown previously as leading to both dilated cardiomyopathy (DCM) and HCM (with sudden infant death).

A carrier of the new V449E variant had a positive family history with SCD. A fourth patient with the HCM-associated polymorphism S236G, had mild HCM with late-onset at 45 y.o. Population analysis of unknown variants (c.2067+38 A>T, V449E), and genetic screening of HCM-associated genes will be further provided in this group of patients.

Conclusion: Genetic screening of HCM-related genes in HCM patients can be used for confirmation of diagnosis, clarifying personal and familial prognosis and genetic counseling.

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P02.123
Regional distribution of mutations associated with hypertrophic cardiomyopathy in the Northwest of Spain

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Background/objectives: Hypertrophic cardiomyopathy has been associated with more than 600 different mutations in sarcomeric genes. Several studies performed in reference centers have reported the presence of multiple families with the same mutation in a given geographic area. Our objective was to evaluate the potential founder effects in a regional population.

Methods: Analysis of the geographic origin of HCM families with mutations in sarcomeric genes identified in Galicia (Northwest Spain).

Results: We have identified 67 different mutations in 376 carriers of 136 apparently independent families (32 MYBPC3 mutations in 61 families, 26 MYH7 in 49 families, 4 TNNT2 in 8 families, 3 TNNI3 in 8 families, 1 TNNC1 in 1 family, and 1 ACTC mutation in 9 families). Forty-four of the 67 mutations (65%) had been previously described. Eighty-eight of the 136 families (65%) shared mutations with other families, and 58 of those 88 (68%) were repeated within the same or a bordering district, strongly suggesting a common origin. Twenty-four of the 67 different mutations appeared in more than one family: 4 were in 2 different families, 9 mutations in 3 families, 3 mutations in 4 families, and 1 mutation was repeated in 5,6, 7,8 and 9 families respectively.

Conclusions: Multiple families with the same mutations are frequent in our population. The knowledge about the geographic origin of the families in a regional setting may be useful to focus the genetic studies. The identification of a high proportion of previously described mutations in multiple families can improve our knowledge about genotype-phenotype correlations.

P02.124

Partial, homozygous deletion of *PREPL* and *C2orf34* on 2p21 in siblings with atypical hypotonia-cystinuria syndrome (HCS)

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Homozygous contiguous gene syndromes are rare. On 2p21, however, two overlapping homozygous contiguous gene syndromes have been described. The first syndrome, referred to as 2p21 deletion syndrome, is characterized by neonatal hypotonia, seizures, severe developmental delay, elevated serum lactate concentrations, poor feeding, growth retardation, facial dysmorphisms and cystinuria type I. The deletion affects at least four genes, PPM1B, SLC3A1, PREPL and C2orf34. The second syndrome, hypotonia-cystinuria syndrome (HCS, MIM606407) is characterized by infantile hypotonia, poor feeding, growth hormone deficiency, mild facial dysmorphism and cystinuria type I; affected individuals carry homozygous deletions encompassing the SLC3A1 and the PREPL gene. Cystinuria is due to the loss of the SLC3A1 gene.

We describe two siblings with a homozygous deletion of 83 kb encompassing exon 1 of the PREPL gene - implicated in both syndromes - and the first three exons of the C2orf34 gene - implicated in the 2p21 deletion syndrome. To our knowledge, this is the first homozygous deletion on 2p21 not disrupting the coding sequence of the SLC3A1 gene. The affected individuals display the classical symptoms of HCS such as severe muscular hypotonia in infancy and pronounced feeding difficulties with growth failure, but no cystinuria. They also show additional features such as cleft palate and genital abnormalities which have not been reported so far, neither in the 2p21 deletion syndrome nor in HCS. Our findings confirm that PREPL is the key gene in causing the complex growth and neuromuscular phenotype in HCS. The role of C2orf34 still remains to be determined.

P02.125

HLA Assessment in Romanian Children Associating IgA Deficiency

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Introduction:Selective IgA deficiency (IgAD) is a genetic disorder characterized by decreased or absent level of serum IgA. IgAD is 10 to 15 times more frequently in patients with celiac disease (CD) than in healthy subjects.IgAD has been associated with chromosomes 18,14 and 6.Certain MHC haplotypes have been associated with susceptibility to IgAD:HLA-A, B14, B8, DR3. Objective: The goal of this study was to assess the association between HLADQ and IgAD in Romanian children. Methods:From 122 celiac patients diagnosed between 2000 and 2010, 9 associated IgAD (diagnosis was IgG serology and histology based).A second study lot consisted in 10 IgAD children without autoimmune associations.The lot of control consisted in 20 healthy children.DQ2/DQ8 alleles were typed by PCR-SSP in all children. Results:Alleles distribution for children with CD and IgAD was:8 cases (89%) presented homozygous DQ2 haplotypes (DQA1*0501/DQB1*0201 and DQA1*0501/DQB1*0201) and one (11%) associated heterozygous DQ8 haplotype (DQA1*0301/DQB1*0302 and DQA1*0301/DQB1*0103).From the second lot IgA deficient without CD, 3children (30%) presented homozygousDQ2 haplotypes.From the contol lot, one healthy children (5%) associated DQ2homozygous alleles. HLA DQ2 homozygous haplotype was present in 58% of total IgAD patients comparing to 5% in controls (p<0,00012).We found a significant correlation between DQ2 homozygous haplotype and IgAD (r=0,62).Conclusions:Presence of HLA DQ2/DQ8 is mandatory but not sufficient for developing CD.Susceptibility to IgAD is associated with DQ2 alleles,but it is not equally in different populations.Differences in HLA association of IgAD reported by various studies suggest that multiple different genes within the MHC region might be involved in the pathogenesis of this immunodeficiency.

P02.126

Mental retardation, microcephaly and cerebellar dysplasia in a child with mosaic interstitial duplication 16q11.2-q12.1

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Intersitial duplications in the long arm of chromosome 16 have been previously reported in a small number of patients. Whereas no distinct common dysmorphic features can be defined for these patients, they all had in common, mental retardation, speech delay and behavioral problems.

We describe a boy who presented with mental retardation, cerebellar signs and mild dysmorphic features who had an interstitial duplication of the long arm of chromosome 16.

This boy is the first child of unrelated parents of Ashkenazi origin. IUGR was noticed at 31w gestation and delivery was induced at 36 weeks due to reduced growth. His birth weight was 1,730 g. He had poor sucking and general psychomotor delay. On examination at the age of 4y, he had microcephally, high nasal bridge, deep set eyes, thin lips, and mild retrognatia. He had low muscle tone and wide-based gait due to clumsiness or ataxia. He used only 5 verbal words and communicated mainly with gestures and signs. Brain MRI revealed cerebellar cortical dysplasia. Large forth ventricle with mild elongated superior cerebellar peduncles and small vermis with atrophy of the inferior aspect were noted.

Microarray and FISH analysis detected a 3.3 Mb mosaic gain from 16q11.2-16q12.2 . and identified a marker chromosome in 17/30 metaphases cells examined. The parents had normal karyotypes.

This is the first report of cerebellar anomalies in a patient with partial trisomy 16q. More patients with an interstitial duplication of 16q11-q12 and brain imaging findings will help to further characterize the phenotype.

P02.127

Report of a Jacobsen syndrome with a mild facial dysmorphism, severe hearing impairment and trombocytopenia

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Jacobsen syndrome (JBS), also known as 11q deletion syndrome, is a rare syndrome with variable phenotypic expression. To date, over 200 cases of JBS have been described, with an estimated prevalence of 1:100000 birth. The most characteristic symptoms are short stature, craniofacial anomalies, congenital heart defects, visceral malformations, developmental delay and thrombocytopenia.

Case report. The proband, 11 months old boy was referred to our department due to suspected genetic disorder. He was born by Cesarean section after a full term pregnancy. His Apgar score was 10-10, birth weight 3000 g, height 50 cm and head circumference 36cm. At the age of 2 months he was hospitalized due to atopic dermatitis and severe foliculitis on the face. Laboratory investigations revealed a low platelet count. Ophthalmological and otolaryngologic evaluation revealed optical nerve subatrophy and severe hearing impairment. Clinical examination revealed short stature, macrocephaly, short neck, ocular hypertelorism, strabismus, epicanthal folds, slight retrognathia, abnormal palmar creases. There were no cardiac, genitourinary and renal anomalies. Cerebral MRI was normal. Slight delay in psychomotor development was noted. According clinical signs diagnosis of Jacobsen syndrome was considered.
FISH analysis using subtelomeric specific probes (ToTelVysion™) for all long and short arms of chromosomes have been performed. Subtelomeric FISH analysis has detected submicroscopic deletion at 11q25. Array CGH analysis for revealing the breakpoints and the size of the aberration is under the way. The present report emphasizes the importance of the FISH as rapid, precise and useful method in such cases with hearing impairment and trombocytopenia.

P02.128
A patient with features reminiscent of Johanson-Blizzard Syndrome and cortical malformations

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Johanson-Blizzard syndrome (JBS; OMIM 243800) is a rare autosomal recessive syndrome, clinically characterised by distinctive facial features, with aplastic or hypoplastic alae nasi, and exocrine pancreatic insufficiency. The clinical spectrum of the disorder include others features as short stature, deafness, mental retardation, scalp defects, oligodontia, hypothyroidism, rectourogenital malformations, ocular abnormalities, heart defects and central nervous system anomalies. Mutations in the UBR1 gene (15q14-q21) are the only genetic cause of JBS known to date.
We describe a boy with a phenotype reminiscent of JBS, in particular facial features, abnormal hair pattern, micropenis and hypopituitarism, and no signs of exocrine pancreas insufficiency at 12 years of age. He also presented an ovalar skull defect (about 2 cm of maximum diameter) in the frontal bone, normal IQ at two cognitive evaluations (5 and 12 years of age) and cortical malformations (nodules of heterotopic gray matter, incomplete development of the right fronto-temporal operculum, dysplastic cortex and mild cerebellar atrophy). The parents reported a previous pregnancy with severe IUGR and hypertrophic cardiomyopathy. The molecular analysis of UBR1 gene did not identify mutations.
The proband could present either a novel condition partially overlapping with JBS, genetically distinct and characterized by a normal pancreatic function, or a variant of JBS phenotype.

P02.129
OFD VI syndrome with cerebellar malformations: clinical, radiological and molecular characterisation

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The oral-facial-digital syndrome type VI (OFD VI) or Varadi syndrome (OMIM 277170) is characterized by the association of malformations of the face, oral cavity and extremities, and distinguished from the 12 other OFD syndromes by cerebellar and metacarpal abnormalities. Cerebellar malformation in OFD VI syndrome has recently been described as a molar tooth sign (MTS), including OFD VI syndrome among the « Joubert syndrome related disorders » (JSRD). We report on the clinical and radiological data of 9 OFD VI cases with normal *OFD1* analysis and molecular investigations of six genes implicated in JSRD. Among the nine collected cases, two were fetuses and 8/9 cases were female. All patients presented oral malformations, facial dysmorphism and distal abnormalities including mainly polydactyly (70%), 50% in hands and 70% in feet. All patients presented with neurological symptoms with mental retardation (5/5 cases), from moderate to severe impairment. Supratentorial abnormalities, brainstem malformations, cerebellar malformations with constant MTS and cystic dilatation of the posterior fossa were always present. In conclusion, the OFD VI syndrome defined by the association of OFD features and cerebellar malformations presents with constant MTS associated to constant brainstem and supratentorial malformation and could definitively be considered as JSRD and an additional ciliopathy. Six of the nine causative genes implicated in JBS and JSRD (*INPP5E; CORS2; TMEM67/MKS3; RPGRIP1L; ARL13B and CC2D2A)* were studied in 5/8 families but no pathogenic mutation was found. The responsible gene(s) probably interact(s) with *OFD1* gene and the JSRD genes.

P02.130
Severe Aortic Stenosis in a Child with Joubert Syndrome and Related Disorders (JSRD) - a Case Report and Review of Congenital Heart Defects Reported in the Human Ciliopathies

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We report a case of a 2 year-old boy with classical features of JSRD including oculomotor apraxia, postaxial polydactyly, episodes of rapid breathing and developmental delay. Magnetic resonance imaging demonstrated the pathognomonic "molar tooth sign". In addition, the child was also diagnosed with severe congenital aortic stenosis and underwent valvular balloon dilatation. The results of gene sequencing of the AHI, TMEM67, CEP 290 and NPHP1 were negative for causative mutation. We are in the process of testing several other genes, including CC2D2A, INPP5E, RPGRIPIL, ARL13B and TMEM216.
JSRD is one of a group of conditions known as 'ciliopathies', whose multi-organ involvement results from primary cilia dysfunction. Human ciliopathies present with distinct but overlapping phenotypes whose core features include retinal degeneration, cystic disease of the liver, pancreas and kidney, postaxial polydactyly and situs inversus. To date, there have not been other reported cases of aortic stenosis associated with JSRD. Indeed, cardiac screening is not currently recommended in the management guidelines for individuals suspected of having JSRD. We speculate that while the presence of congenital aortic stenosis in this child could be caused by an unrelated genetic mechanism, it could also represent a phenotypic overlap with another ciliopathy, the Bardet Biedl syndrome, in which aortic stenosis is present in about 38% of cases.
We also review the range of cardiac lesions reported to be present in all human diseases known to be ciliopathies, in order to assist with the investigation and management of individuals with a suspected or proven ciliopathy.

P02.131
Neuroimaging, clinical and genetic analysis of novel and known congenital neurodevelopemental brain malformations followed by deep sequencing

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Intellectual disability (ID) occurs as a symptom in heterogeneous groups of neurodevelopemental disorders.
Three of the most important contributions in understanding of the structural or functional brain defects are the capacity to perform detailed neuroimaging, the ability to perform highly advanced genomic testing and the accurate and professional characterization of the patients. Neuroimaging could provide more refined correlations between clinical and molecular evaluations, and could result in to the identification of genetic causes of nurodevelopemental brain diseases.
We have investigated one hundred mentally retarded patients clinically candidate for neurodevelopental brain malformations .Up to now we have performed neuroimaging for 60 families, which for some of them next generation sequencing have been recently done to identify genetic defects as well.
We could identify 15 families with cerebellar hypoplasia or atrophy, two families with Joubert syndrome type3 (with mutations in AHI1), two families with CP like syndrome and mutations in *AP4A1* as a recently identified syndrome and two families with spinocrebellar ataxia type 14 syndrome (with mutations in PRKCG).
Our findings suggest that more than thirty percent of autosomal recessive ID (ARID) families with neurologic symptoms suffer from different types of neurodevelopemental brain malformations.

P02.132
Microdeletion 10q23 and juvenile polyposis syndrome: a case report

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Juvenile polyposis syndrome is a rare disease characterized by hamartomatous polyps in the gastrointestinal (GI) tract. For clinical diagnosis there has to be one of the three following findings: more than five juvenile polyps of the colorectum; multiple juvenile polyps throughout the GI tract; any number of juvenile polyps and a family history. Approximately 20% of individuals with JPS have mutations in *BMPR1A* and approximately 20% have mutations in *SMAD4*.
We describe a 4-year-old boy who was born with congenital heart defect (atrioventricular septal defect), macrocephaly and dysmorphic features. His karyotype from lymphocytes was normal (46,XY). According to his clinical picture (facial dysmophism, failure to thrive and heart anomaly) Noonan syndrome was clinically diagnosed at the age of 1 year although no mutations in *PTPN11* and *KRAS* genes was detected. At the same age the iron deficiency anaemia was diagnosed and in the second year of life he has blood in the stool. At the age of 4 years polyps were found all over the GI tract. His development was delayed, he was dysmorphic with macrocephaly (+4 SD), in MRT of the brain showed mild ventriculomegalia. There was a suspicion to 10q microdeletion according to his clinical picture. Whole-genome genotyping was performed, using the Illumina platforms and 5,1Mb deletion in the region 10q23.1-23.3 was found. This finding was confirmed also by quantitative PCR analysis. In 10q23.1-23.3 region both *BMPR1A* and *SMAD4* genes are located so the deletion is responsible to the polyposis of the patients.

P02.133
Extended genotype - phenotype correlation study in Kabuki syndrome patients comparing MLL2 mutation carriers vs. non-carriers

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Kabuki syndrome (KS, MIM: 147920) is a clinically recognizable syndrome of multiple congenital anomalies and mental retardation affecting approximately 1:30,000 live births. Key features are a characteristic face, growth retardation, developmental delay and additional features such as hypodontia and persistent foetal fingertip pads. Recently, a gene causing KS was identified through exome sequencing, reporting de novo mutations in the histone methyl transferase (HMT) gene *MLL2* in 66% of 53 patients with Kabuki syndrome (Ng et al., Nat Genet.42(9):790-3, 2010). We confirmed the pathogenic significance of this gene in KS in our first published series, demonstrating 76% MLL2 mutations in 45 KS cases (Paulussen et al, Hum Mutat. 32(2), 2011). In another series of 31 patients, we identified 21 additional novel MLL2 mutations. Thus the combined mutation yield of Sanger-based MLL2 exon sequencing for both series is 55/76 = 72%, further supporting the major contribution of MLL2 mutations to the disease. We collected detailed clinical data of the 76 patients from both series (49 female, 27 male). Relevant clinical features were selected from the Dyscerne guidelines for KS (www.dyscerne.org) and interrogated using standardised questionnaires and checklists. Analysis focused on 7 clustered feature groups: facial, growth and development, cardiovascular, sensory (vision, speech, hearing), skeletal/orthopaedic malformations and accessory minor feautures. Statistical analysis using SPSS v12 was used to correlate (clustered) clinical features to MLL2 mutation carrier status We will present data from an extended pheno-genotype analysis, including the contribution of type-specific clustered mutations (i.e. nonsense, frame-shift, missense and splice-site).

P02.134
"Kabuki make-up syndrome" - characterization of rare findings of 2 new patients

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"Kabuki make-up" syndrome (KS, MIM 14792) is a clinically distinct MCA/MR disorder with uncertain inheritance (most cases are sporadic). The main diagnostic criteria are well-known, but a phenotypic spectrum of rare abnormalities have delineated. We present clinical pictures of 2 new unrelated patients and characterize rare features in comparison with literature. Patients were a single child of young healthy nonconsanguineous couples. Pregnancy, labor were unremarkable. Growth delay, mental retardation, craniofacial dysmorphisms, heart involvement, fingertip pads were common. Karyotype (GTG): 46,XX; 46,XY. Metabolic disorders, prenatal infections were excluded. Case 1: Girl, 2 years old, showed typical facial appearance, dysmorphic ears, preauricular fistulas, lower palpebra eversion, cleft palate, diaphragmatic hernia, heart defect (ASD,VSD). Case 2. 12 years old boy presented severe mental and speech delay, tetraparesis, brain cyst, large prominent malformed ears, hearing loss, arched eyebrows, long palpebral fissures, nystagmus, strabismus, bilateral coloboma of iris, chorioidea and optic nerves, myopia, cataracts, high palate, malocclusion of teeth, scoliosis, thorax deformation, flat feet. Heart defect (AVC, VSD, insufficiency of valves). At the time of counseling mother was pregnant and underwent to standard combined prenatal screening. No fetal malformations were detected. Families were reffered for genetic counseling due to affected child for diagnostics and prognoses. We have established KS (sporadic origin) based on association of characteristic facial features, growth and mental delay. Uncommon malformations were preauricular fistulas, cleft palate, diaphragmatic hernia (case 1), ophtalmological abnormalities, scoliosis (case 2). Rare signs could not influence for diagnostics if main criteria were found.

P02.135
MLL2 gene sequencing in a series of 79 patients with Kabuki syndrome.

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Kabuki syndrome (KS, MIM 147920) is a MCA-MR syndrome characterized by a facial gestalt, intellectual disability, visceral and skeletal malformations and, postnatal short stature with overweight.

An exome sequencing strategy recently identified *MLL2* as the disease-causing gene with heterozygous, *de novo* CDS mutations in about 70% of the patients fulfilling clinical diagnostic criteria.

Here we report the identification of an *MLL2* mutation in 80% of the cases from a series of 79 KS patients. All patients were sporadic except for one KS familial case with two sibs and their mother being affected. Mutations are spread throughout the gene with no hot-spot. Nonsense, frameshift ,splice site, Missense and in frame duplication/deletion mutations were found in 15 (23%), 27 (42%), 2 (3%) 18 (28%), and 2 (3%) patients respectively.

Phenotype/genotype correlations were investigated for intellectual disability, growth, seizure, cleft lip/palate, cardiac and renal malformations, endocrine disorders (GH deficiency and hyperinsulinism), and dysimmunity (immunoglobulin deficiency and auto-immune manifestations).We also compared patients with and without *MLL2* mutations in an attempt to delineate clinical, biological and skeletal features allowing to differentiating the 2 groups.

P02.136
A novel *de novo* splice-site mutation in MLL2 gene in a patient with Kabuki syndrome

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Kabuki syndrome (KS, MIM# 147920) is a rare disorder characterized by developmental delay, distinctive facial features and short stature. Recently, Ng et al. identified mutations in MLL2 gene as causative of KS. Up to date, approximately 65 mutations have been described in MLL2 gene related to KS, being most of them truncating mutations with a *de novo* origin.

In this work, we describe the case of a 14 month old female patient referred to our Hospital in order to study her developmental delay and growth retardation. She presented microcephaly, ventricular septal defect, hypotonia and joint laxity, persistence of fetal finger pads and a sacral dimple. She showed facial features as arched eyebrows with sparseness of hairgrowth in the lateral half, long eyelashes, long palpebral fissures with an one third of the lower lid everted lateral, broad nasal tip and prominent ear lobes (anteverted and with simplified morphology). She also showed feeding difficulties and breast development from 3 months of age.

In order to confirm the clinical suspect of KS we performed complete sequencing of MLL2 gene. This genetic study revealed the presence of a splice site mutation, not previously reported in the literature. De novo origin of the mutation was confirmed due to its absence in patient's parents. Currently, RNA studies are being carried out to determine the causal mechanism of the mutation.

P02.137
A large mutation screen in patients with Kabuki syndrome

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Epigenetic control of developmental processes is a fascinating mechanism by which spatial and temporal expression of distinct genes and pathways is regulated. Alterations of epigenetic mechanisms have been mainly associated with the pathogenesis of cancer, but recently also with the occurrence of congenital malformation syndromes. An up-to-date example is the identification of *de novo* dominant mutations in the *MLL2* gene using a whole-exome sequencing strategy in patients with Kabuki syndrome. Kabuki syndrome is a classical, clinically well-known multiple congenital anomalies/mental retardation syndrome, mainly characterized by a distinct facial appearance in addition to e.g. developmental delay, short stature, persistent fingerpads, and urogenital tract anomalies. In our study, we sequenced all 54 coding exons of the *MLL2* gene in 34 patients with Kabuki syndrome. We identified eighteen distinct mutations in nineteen patients and twelve of thirteen tested *de novo*. Mutations included four nonsense mutations, two splice-site mutations, six small deletions or insertions, and six missense mutations. The mutations were located throughout the *MLL2* gene without a clustering in specific exons of the gene or domains of the protein. Interestingly, genotype-phenotype comparison indicated a higher frequency of specific symptoms in *MLL2*-positive cases. Mutation-negative patients were subsequently tested for mutations in 12 highly relevant functional candidate genes (e.g. *MLL1, ASC2, ASH2*, and *WDR5*) and we identified one alteration in *MLL1* with a yet unclear causative role. Our results indicate that *MLL2* is the major gene for Kabuki syndrome with a wide spectrum of *de novo* mutations and they provide evidence for further genetic heterogeneity.

P02.138
Is Hardikar syndrome distinct from Kabuki (Niikawa-Kuroki) syndrome? Report of a case with a novel mutation in the MLL2 gene.

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Kabuki syndrome (Niikawa-Kuroki syndrome) is characterised by distinctive facial features, skeletal anomalies, dermatoglyphic abnormalities, mental retardation, and growth deficiency. Recently, mutations in the MLL2 gene were identified as the genetic cause of Kabuki syndrome. Hardikar syndrome is a rare condition of unknown cause in which cleft lip and palate, patchy pigmentary retinopathy (''cat's paw'') and obstructive liver disease are associated.

We describe a patient with typical facial features of Kabuki syndrome who also developed a hepatoblastoma and retinal pigmentation anomaly cat's paw type. The pathogenic c.11119C>T nonsense mutation in the exon 39 of MLL2 was found. By comparing our patient's phenotype with the patients with Hardikar syndrome described to date, we focussed on the overlapping features of the two syndromes. As a conclusion, we suggest that Hardikar syndrome could be an extreme of the wide phenotypic spectrum of Kabuki syndrome.

P02.139
A case with Kallmann syndrome and ichthyosis: not always a contiguous gene syndrome

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Kallmann syndrome consists of congenital, idiopathic hypogonadotropic hypogonadism and anosmia. The X-linked form is caused by mutations in the *KAL1* gene on chromosome Xp22.3. Some patients have larger deletions encompassing neighbouring genes and thus may present with other clinical features, such as X-linked ichthyosis (XLI), which results from steroid sulfatase deficiency due to loss of function mutations of the STS gene at Xp22.3.

We report a 9 year and 7 month old boy who was diagnosed with

ichthyosis and Kallmann syndrome on the basis of hypogonadotropic hypogonadism, left kidney agenesis and hyposmia. Close examination of the patient's family history revealed an apparently X-linked pattern of ichthyosis with three affected male relatives of the mother. On examination auxological parameters were normal. Skin appeared dry and scaly, particularly at limbs, ears, trunk and feet. His parents and younger brother were phenotypically normal, without detectable renal anomalies on ultarsounds.
Array-CGH analysis on peripheral blood lymphocytes detected a deletion of about 500 kilobases, involving the 5' region of the *KAL1* gene, but not the *STS* gene; the same deletion was then confirmed in the proband's mother. Quantitative PCR analysis of the *STS* transcript showed normal expression levels and sequencing of the whole coding region of the *STS* gene did not reveal any sequence variant.
Our excludes a potential positional effect of the genomic rearrangement on the expression of the *STS* gene and supports the hypothesis of additional loci for ichthyosis on the X chromosome.

P02.140
Lafora disease with a new inheritance

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A type of progressive myoclonic epilepsy called Lafora Disease (OMIM 254780) is a very rare autosomal recessive fatal genetic disorder caused by mutations in laforin gene or malin gene. It is described as starting in early adolescence as grandmal seizures and/or myoclonus and characterised by the presence of periodic acid-Schiff-positive staining intracellular inclusion bodies. We report a patient diagnosed as Lafora disease with a possibility of an autosomal dominant inheritance.
A 25 years old girl reffered from neurology clinic to genetic clinic for pedigree analysis who has myoclonic and grandmal tonic-clonic seizures started at age 15, dysarthria, ataxia, visual hallucinations and depression. Intracellular PAS positive polyglucosan inclusion bodies (Lafora bodies) has been showed in skin. Pedigree analysis revealed an autosomal dominant inheritance which is not described in literature before. Patient's parents aren't consanguineous. Her father, aunt and grandfather diagnosed as Lafore,too. So, we suggest that a new gene or mutation may cause Lafore in this family . Further studies will be done for highlighting the reason of this unexpected inheritance.

P02.141
Langdon-Down syndrome in a newborn from mother with epilepsy - Case report

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Introduction: Down syndrome is the most common and known chromosomal disorder.
This syndrome is characterized by mental retardation, facial dimorphism and other phenotype distinctive traits.
Many individuals with Down syndrome have also heart defects.
Objective: Describe a case of Down syndrome from mother with severe epilepsy under treatment.
Material and methods: Medical history: the patient is a 2.500 gram male newborn, GIV, PIV, delivered by cesarean section at 38 weeks of gestation; his 38-year-old mother is known with: Severe epilepsy partially controlled by medication - 4 seizures/month (she was treated throughout pregnancy); his father is 41-year-old.
Physical examination admission revealed: serious general condition, short neck, facial dismorphism: hypertelorism, upward slanting eyes, epicanthal folds, low set ears; perioral cyanosis; short hands; hypospadias; poor muscle tone, poor reflexes; systolic murmur grade III/VI.
Cytogenetic analysis showed 100% of 21 trisomy; karyotype: 47XY, +21
Imagistic examination
- Echocardiography: hypertrophic cardiomyopathy; atrial and ventricular septal defect; aortic stenosis and tricuspid insufficiency.
- transfontanelar ultrasound : hypoxic ischemic encephalopathy.
Results: During hospitalization evolution were wavy; gaining slowly in weight; abnormal development: the absence of sucking reflex (gavage feeding); poor muscle tone; he required antibiotics for respiratory problems, cardiologic and neurological treatment.
Conclusions: The prognosis is variable, depending on the types of complications.
Increased incidence of cardiac malformations requested cardiologic consult in every case because congenital heart disease is the major cause for early mortality.
The development and growth is usually delayed and often average height and developmental milestones are not reached.

P02.142
Laryngomalacia (OMIM 150280): unexpectedly high frequency in population

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The diagnosis of laryngomalacia is based on endoscopic revealing of inspiratory collapse of laryngeal vestibule. Many investigators proved this disorder as the most common congenital malformation of upper airways. Despite this, no one medical geneticist reasonably regards this diagnosis as real genetic syndrome "laryngomalacia" (OMIM 150280). No data on frequency of this syndrome in population appeared to be found in world literature.
Complex investigation in three groups was performed: 369 children (1 to 38 months old) with congenital stridor (separation of genetic syndrome), 10 children with laryngomalacia and no surgical treatment (follow up more than 5 years), 300 adults with absence of laryngeal problems in anamnesis (assessment of laryngeal vestibule).
Identical findings, most close to genetic syndrome "laryngomalacia", were obtained in 77 cases of congenital stridor. They include typical stridor, inspiratory supraglottic collapse, anatomic anomalies of laryngeal vestibule, any of related pathology (gastroesophageal reflux, failure to thrive, obstructive sleep apnea, chokes), typical course, family character (the last was found just in 27 cases). All cases with prominent catarrhal changes, neurological problems, other congenital malformations of upper airway were excluded. Thereby, "laryngomalacia" (as syndrome) provides more than 40 % events of congenital stridor.
Ten cases of laryngomalacia were followed up till anatomic peculiarities of laryngeal vestibule became stable (children reached 7 to 9 years old) and tried to find the same changes in 300 healthy adults. It had been successful in 13 (4 %) cases, that represents minimal frequency of syndrome "laryngomalacia" in population.

P02.143
Leber Congenital Amaurosis (LCA): development of a comprehensive molecular genetic test panel using next-generation sequencing

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Leber Congenital Amaurosis (LCA) is associated with 16 genes, accounting for 70% of cases. Generally, genetic testing is limited to screening of a selected number of mutations and/or Sanger sequencing of a subset of genes. We aimed to design an accurate, fast and affordable molecular test for all known LCA genes using next-generation sequencing.
We developed a novel protocol consisting of qPCR, ligation, fragmentation, sequencing on an Illumina GAIIx, and data analysis by NextGENe. As a proof-of-concept, ten LCA patients were included, five of which had a known molecular defect.
Using in-house primer design, 375 amplicons were developed to cover 236 exons and intron-exon boundaries of 16 genes (*RD3*, *RPE65*, *CRB1*, *MERTK*, *IQCB1*, *LRAT*, *LCA5*, *TULP1*, *IMPDH1*, *CEP290*, *RPGRIP1*, *RDH12*, *SPATA7*, *AIPL1*, *GUCY2D* and *CRX*). Following amplification, ligation and shearing, amplicon pools were sequenced in one GAIIx lane (1x100bp, indexing). This yielded sufficient coverage for 93-95% of exons. In total, 104/107 previously identified variants were detected, including the known mutations in the positive controls. In addition, mutations were identified in 2/5 mutation-negative patients.

In conclusion, we developed a cost-efficient workflow for parallel sequencing of all known LCA genes, which enables sequencing of regions with variable length, such as exons, on a short-read sequencer. The flexibility of this approach allows easy expansion with other genes, providing an excellent basis for molecular testing of other retinal dystrophies. Finally, our protocol enables an early molecular diagnosis for LCA, which is essential with respect to reproductive issues, prognosis and eligibility to gene therapy.

P02.144
New primary mutations in Iranian LHON patients

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Leber Hereditary Optic Neuropathy (LHON) is a maternally inherited blinding disease caused by missense mutations in the mitochondrial genes that encode subunits of NADH dehydrogenase (complex 1 of respiratory chain), include MT-ND (1-6). It is characterized by bilateral, painless, subacute visual failure that develops during young adult life. Sequence analysis of the all ND genes was performed in 30 Iranian patients with clinical symptoms of LHON. The result showed that 20% only have primary mutations (3460G>A, 11778G>A, 14484T>C) while 17% just present secondary mutations (4216Y>H, 4917N>D,...) ,of which only 6% have both types of mutations. 50% of patients with primary mutations showed 11778G>A while 33% have 3460G>A and 16% present 14484T>C mutation. Also 17% of patients are lack of any mutations and even polymorphisms and finally 46% revealed new homoplasmic mutations (9949V>I, 13802T>I, 3893T>S) which were not reported before and not existed in LHON patients with primary or/ and secondary mutations. Because the mitochondrial genome contains a large number of apparently neutral polymorphisms that have little pathogenic significance, along with secondary homoplasmic mutations that do not have primary disease-causing effect, the pathogenic role of all newly discovered mutations must be rigorously established with familial assay, investigation of nuclear genes and evaluation of other diseases which have common symptoms with LHON . We believed that these new mutation may have primary effect in LHON.

P02.145
Molecular-clinical correlation in a family with a novel heteroplasmic Leigh syndrome missense mutation in the mitochondrial cytochrome c oxidase III gene

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The clinical spectrum of mitochondrial disorders is extremely heterogeneous, affecting young and adult patients at any age, with involvement of a wide variety of tissues. More than 100 mitochondrial DNA (mtDNA) mutations have been described in association with different neurological disorders and with respiratory chain deficiency. Besides the most frequently reported mitochondrial tRNA mutations and deletions, pathogenic mutations affecting structural genes of mtDNA-encoded respiratory chain subunits, mainly cytochrome b and cytochrome c oxidase (COX) subunit genes, have been reported in association with various mitochondrial disorders such as Leigh syndrome. Cytochrome c oxidase is an essential component of the mitochondrial respiratory chain that catalyzes the reduction of molecular oxygen by reduced cytochrome c. In this study, the authors report the second mutation associated with Leigh syndrome in the blood and buccal mucosa of 2 affected members of a Tunisian family. It was a novel heteroplasmic missense mitochondrial mutation at nucleotide 9478 in the gene specifying subunit III of cytochrome c oxidase substituting the valine at position 91 to alanine in a highly conserved amino acid. It was found with a high mutant load in tissues derived from endoderm (buccal mucosa) and mesoderm (blood). However, it was nearly absent in tissue derived from ectoderm (hair follicles). It was absent in 120 healthy controls, and PolyPhen analysis showed that the hydropathy index changed from +1.276 to +0.242, and the number of structures of the 3D protein decreased from 39 to 32.

P02.146
Leigh syndrome in two Iranian sisters with mild phenotype with novel missense mutation in SURF1

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Leigh syndrome (LS) is a rare inherited neurometabolic disorder that affects the central nervous system. This rapidly progressive disorder usually occurs in infants while rarely in teenagers and adults. LS is a heterogeneous condition with at least 3major causes , each defferent modes of inheritance; X-linked, autosomal recessive and mitocondrial. SURF1 locus is identified to be affected in LS patients. Insertion/ delition and missense/ nonsense mutations in exons and splicing sites in introns have been detected in LS patients. Missense mutations have been reported to cause mild LS. Symptoms include hypotonia, ataxia, seizures, brain stem and basal ganglia dysfunction, spasticity and ophtalmoplegia. Generalized weakness and episodes of lactic acidosis may also be diagnosed.

We report a novel missense mutation in SURF1 locus (c.679T>C,W227R) in two iranian sisters ages 25 and 27 years old with ataxia (started from 3 years of age), disarthria, short stature and normal cognition. To the best of our knowledge they are the longest survivng LS patients.

P02.147
LMNA mutation in Hutchinson-Gliford progeroid syndrome in association with strokes

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Hutchinson-Gilford progeria syndrome is a very rare but well characterized genetic disorder that causes premature ageing. The clinical features affect growth, skeleton, body fat, skin, hair and the cardiovascular system. It is caused by mutations in *LMNA* gene, the most frequent being p.Gly608Gly (c.1824C>T) in exon 11.

Here we present a four-year-old HGPS patient presenting a heterozygous *LMNA* missense mutation in exon 2. This mutation is located far from the C-terminal region implicated in the posttranslational processing of prelamin A, but it lies within the rod domain of lamin A/C that represents a highly conserved domain specific to nuclear lamins. We hypothesize that this region could be involved in early and severe strokes in HGPS, as those presented by our patient. This is the first report of an HGPS patient with Maghrebian origin.

P02.148
Severe Lysosomal Storage Disease of Liver in del(1)(p36): A New Presentation

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1p36 deletion is the most common terminal deletion syndrome with an estimated occurrence of 1:5000 live births. The deletion is of variable size. It usually involves less than 10 Mb in the 1pter-1p36.23 interval. Variability of the phenotype is partially related to the extent of the deletion. Some children with a 1p36 deletion were reported with obesity and hyperphagia, raising the question of possible phenotypic overlap with Prader-Willi syndrome. Correlation between presence of obesity and the size of the deletion has only be documented in one case. We report an 11-year-old girl with 1p36 deletion and the classical dysmorphological features. In late infancy, she developed an uncontrolled voracious appetite, overweight, truncal obesity and elevated serum transaminases. Liver biopsy disclosed severe steatosis. The hepatocytes contained accumulation of lipofuscins. Lipolysosomes were abnormally numerous and extremely enlarged. These features have not been previously reported in 1p36 deletion. Oligonucleotide-based microarray analysis showed a subtelomeric 2.2

Mb deletion at 1p36.33p36.32. This suggests that this chromosome segment is a critical region for obesity and hyperphagia. The accumulation in the liver with abnormal ultrastructure may be an additional feature of this form of syndromal obesity. 1p36 deletion syndrome should be considered in patients with obesity, hyperphagia and liver fat accumulation.

P02.149
Mabry Syndrome: the evidence for genetic heterogeneity

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Mabry syndrome (hyperphosphatasia with developmental disability) was first described by Mabry et al. in 1970 a single family (OMIM#239300) [1970]. At first considered rare, our work to describe the disorder has led to recruitment of over twenty families world-wide. Descriptions of this cohort have resulted in the recognition of several other salient features of the disorder including: a characteristic facial dysmorphology (hypertelorism, a broad nasal bridge and a tented mouth); subtle hand bone abnormalities (variable shortening of middle and distal phalanges) and nerve abnormalities (plaques disrupting Schwann cells). Like many infantile metabolic storage disorders, seizures associated with Mabry syndrome have an onset in the first year of life. Persistently elevated serum alkaline phosphatase (ALP) levels are now known to be associated with abnormalities of the phosphoinositol glycan (PIG) anchor, which was found to be disrupted in eight of these families. Lysosomal storage material detected in patients with Mabry syndrome has been putatively identified as glycolipid material. As PIGV mutations have not been identified in all patients with a clinical diagnosis of Mabry syndrome, we describe this cohort. As twenty genes are integral to PIG anchor synthesis, PIGV mutations negative patients or those with vacuolar plaques may result from disruptions to other genes in the PIG anchor biosynthesis pathway. This work will assist in elucidating the inborn errors of metabolism that underlie Mabry syndrome.

P02.150
Identification of an additional putative Brunner syndrome family in Southern Sweden by means of genomic microarray analysis

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We have identified a Swedish family with a partial deletion of the MAOA gene located on the X-chromosome. MAOA (monoamine oxidase A) is involved in the degradation of neurotransmitters. The detected deletion presumably leads to a complete lack of functional MAOA in male carriers.

The proband in the present study is a 13-year-old boy who was subjected to genomic microarray analysis using the Affymetrix Cytogenetics Whole-Genome 2.7M Array due to a history of behavioral problems including social interaction and slow learning. His intelligence has been classified within normal range, but he has a diagnosis of ADHD with symptoms in line with DAMP (deficit of attention, motor control and perception). He is obese with BMI 33,8. The proband also has a brother with a diagnosis of ADHD.

We detected a 1.2 Mb deletion on Xp11.3-p11.4 in the proband, including the first three exons of the gene MAOA as the only known gene. An identical deletion was detected in the boy's mother. Functional analyses and extended characterization of the family is ongoing.

This study describes the first kindred with an isolated functional abrogation of MAOA since 1993, when Brunner et al. reported a Dutch nondysmorphic kindred with borderline mental retardation, prominent behavioral disturbances and an inactivating point mutation in MAOA. The behavioral problems noted in our proband seems similar to the symptoms in the Dutch kindred. The subjects in the original family were not obese, but other studies have suggested an association between low MAOA activity and increased BMI.

P02.151
Marfan syndrome: role of inflammation in dilatation or dissection of the aorta

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Background. The primary cause of early death in untreated Marfan syndrome (MFS) patients is aortic dilatation and dissection. A defective gene causing MFS, FBN1, encodes for an extracellular matrix protein called fibrillin-1, a component of the elastic fiber system. The fibrillin-1 deficient mouse, an accepted model of MFS that recapitulates the cardiovascular phenotype, shows medial degeneration in the aortic wall that was surprisingly associated with inflammatory infiltrates prior to dilatation or dissection.

Methods. We examined 13 patients with MFS after aortic valve-replacing root operations. Full aortic segments and blood samples were collected from patients undergoing repair. Immunohistochemistry staining was performed using antibodies directed against markers of lymphocytes and macrophages.

Results. Immunohistochemisty demonstrated that the media and adventitia had increased numbers of T lymphocytes and macrophages, indicating the role of inflammation in the onset and progression dissection of the aorta. Macrophages were significantly increased in the aortic media with infiltrated aneurysms. CD3+ cells were localized in the media and surrounding the vasa vasorum in the adventitia. Transmural inflammation was associated with increased aortic external diameter, intimal thickening, preserved vascular smooth muscle cell density, and decreased matrix proteins. We found elevated level of TNF-α, IL-2, IL-6, IL-8 in 12 patients.

Conclusions. These results indicate that infiltration of inflammatory cells contributes to the pathogenesis of MFS. Immune mediated aortitis may contribute to pathogenesis of dilatation or dissection of the aorta in MFS formation involve inflammatory infiltrates in the aortic media. Superantigen-driven stimulation of T lymphocytes may contribute to the initial immune response.

P02.152
McCune-Albright syndrome - Importance of active screening for complications

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McCune-Albright syndrome (MAS) is classically defined by the clinical triad of fibrous dysplasia of bone, café-au-lait skin spots, and precocious puberty. MAS is a rare disease with estimated prevalence between 1/100,000 and 1/1,000,000. We present a case of a 9 year old girl who shows deformations of the hand bones, 2 café-au-lait skin spots and development of breast tissue. Her height and weight is normal for age, wihle the bone age is slightly advanced.The endocrinological examination showed elevated prolactin and estrogen, with other hormones in normal range. All other investigations are in normal range. GH (growth hormone) and prolactin excess are common (21%), but the signs and symptoms can be very subtle. Most GH secreting tumors are co-secretors of GH and prolactin, GH excess can worsen craniofacial bone disease and hyperprolactinemia can independently have an adverse affect on gonadal function. Consequently, it becomes important to assess prolactin level and have an oral glucose tolerance test to look for non-suppressible GH at least once, in order to make the diagnosis and treat.

P02.153**

Meier-Gorlin syndrome: Genotype-phenotype characterization of 23 patients with mutations in five separate genes of the pre-replication complex

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Meier-Gorlin syndrome (MGS) is an autosomal recessive disorder characterized by microtia, patellar aplasia/hypoplasia and short stature. Additional common features encompass a characteristic facial appearance, lung and genitourinary malformations.

Recently, we identified mutations in five genes from the pre-replication complex, crucial in cell cycle progression and growth, in 23/38 (61%) individuals with MGS.

Here, we report on genotype-phenotype studies in this unique cohort (13 females, 10 males; age 3 months - 47 years). The triad of microtia, absent/hypoplastic patellae and short stature was observed in 60% of individuals. Microtia and short stature with normal patellae was seen in 15%; microtia and abnormal patellae without short stature in 15%; short stature only in 10%.

Intrauterine growth retardation was present in 76%, postnatal growth retardation in 86% and microcephaly in 50%. Compound heterozygous or homozygous mutations were detected in *ORC1* (39%), *ORC4* (13%), *ORC6* (13%), *CDT1* (31%) and *CDC6* (4%). Seventeen different mutations were identified: ten missense, three nonsense, two frameshift, two splice-site mutations.

Pre-replication complex mutations appear to be associated with a broader phenotypic spectrum than previously expected. No clear genotype-phenotype association was observed, although compound heterozygous frameshift mutations seem to give rise to a more severe phenotype compared to homozygous mutations, particularly in *ORC1* and *ORC4*. Homozygous missense mutations in *CDC6*, however, resulted in more severely impaired growth than compound heterozygous nonsense mutations in *CDT1*. Further studies in additional individuals are needed to substantiate these observations and to understand the role of the separate genes in normal and disrupted organ development.

P02.154

Audiological caracteristics of syndromic and nonsyndromic patients carrying mtDNA 3243A>G mutation

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Background: Patients harbouring the 3,243A>G mutation in tRNA leucine (UUR) gene (MTTL 1) frequently develop multisystemic disease, however they may present an isolated sensorineural hearing loss (SNHL).

Aim: To compare audiological parameters of syndromic and nonsyndromic patients carrying the 3,243A>G mutation.

Methods: Study group include 32 patients (16 probands) carrying the 3,243A>G mutation. We analysed DNA samples isolated from blood leukocytes, hair follicles, urinary sediments, buccal mucosa and nails of each patient.

Molecular search for the 3,243A>G mutation was performed on RealTime PCR using TaqMan Assay; a heteroplasmy level was assessed using PCR-RFLP analysis.

Results: Among 16 nonsyndromic patients (11 women), aged from 6 to 62 yrs; mean age 19 yrs postlingual, stable SNHL or normal hearing was frequently observed.

17 syndromic patients (10 women) aged from 12 to 75 yrs; mean age 39 yrs mainly present poslingual progressive SNHL or retrocochlear hearing loss. Tinnitus was reported by five, vertigo by six patients.

Maternal inheritance was revealed in all families but one.

Conclusions: Patients with postlingual isolated SNHL demonstrating maternal inheritance require a diagnosis for the 3243A>G mutation. Progressive postlingual SNHL in syndromic patients seems to be a good phenotypic marker of mitochondrial disorder.

P02.155

Evidence of germinal mosaicism associated with *MEN1* gene deletions: implications for genetic counseling

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Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterized by combinations of more than 20 endocrine and non-endocrine tumours. The penetrance is 95% by age 40 years. Germline *MEN1* mutations are identified in about 85% of individual with familial MEN1 syndrome, large deletions are rare. We report 2 families with MEN1 gene deletions and evidence of germinal mosaicism.

Family 1: MEN1 was diagnosed in a 30-y-o male. Family history was negative. *MEN1* sequence analysis failed to detect any mutation. Few years later, his 54-y-o father was referred for MEN1. Quantitative analysis of *MEN1* in the proband showed a heterozygous deletion of exons 2-10. Father's blood and saliva DNA analysis was repeatedly negative. The mutation was present in tumor sample. Predictive genetic testing in proband's siblings identified one 30-y-o carrier.

Family 2: MEN1 was diagnosed in two adolescent sisters. *MEN1* analysis failed to detect any mutation. Several years later, quantitative analysis of *MEN1* showed a heterozygous deletion of exons 1-6. At that time, family history revealed a paternal uncle was treated for HPT. Genetic analysis showed that he and the asymptomatic 45-y-o father carried the exons 1-6 *MEN1* deletion. QMPSF in their mother sample showed ratio compatible with mosaicism.

MEN1 somatic mutation is assumed in sporadic MEN1 cases with negative blood analysis. To our knowledge, we present the first evidence of germinal mosaicism in MEN1. In both cases, *MEN1* mutations are deletions events, associated with a lower penetrance. This observation raised questions regarding genetic counseling in MEN1 families.

P02.156
Approach to molecular screening in Polish patients with hearing loss

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Background: In European countries, about 2/1000 children suffer from severe congenital or prelingually hearing loss, another 1 / 300 children are born with a minor degree of hearing loss. Causes of hearing loss are complex and in most cases harbor a combination of genetic and environmental factors. It is assumed that approximately 60% of isolated cases of hearing loss are genetic. For this type of hearing loss most often are responsible mutations in GJB2 and GJB6 genes. The optimal diagnostic algorithm for this group of patients can reduce the cost of conducting effective research and genetic counseling.
Aims: The aim of the study was to present an algorithm of molecular investigations in patients with a suspicion of a genetic based hearing loss.
Materials: 6882 DNA samples retrieved from patients suffering from bilateral hearing loss prelingual and postlingual, varying in degree from mild to severe. Only non consanguineous patients (probands) were included for the analysis.
Methods: Mutliplex PCR test for 5 common Polish GJB2 mutations, direct sequencing of coding exon of GJB2 gene, rtPCR test for the presence of IVS1+1G>A mutation, AS-PCR for the detection of GJB6 D13S1830 deletion, real time PCR for the detection of MELAS 3243 A>G mutation.
Results: Molecular analysis, performed step by step, revealed presence of the two pathogenic mutations in a group of 720 among 4103 analyzed probands.
Conclusions: Application of the optimal algorithm of molecular tests can detect the cause of hearing impairment in about 18% of patients.

P02.157
Detection and assessment of mitochondrial DNA heteroplasmy in patients with MELAS disease

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Mitochondrial encephalomyopathy, lactic acidosis and strokelike episodes -MELAS, is one of several mitochondrial diseases. Clinical features also may include: short stature, seizures, episodic vomiting, sensorineural hearing loss and others. Symptom onset can occur protean, also incomplete or milder phenotypes are recognized.
We studied 1478 Polish subject recruited from a consecutive cohort of 7000 treated at the Institute of Physiology and Pathology of Hearing between 2000 and 2010. Patients were unrelated and suffered from nonsyndromic, postlingual, bilateral sensorineural HI ranging from mild to profound. All individuals were previously tested for the presence of common GJB2/GJB6 mutations.
Searching for the mutation A3243G was performed using different molecular techniques (direct sequencing, RealTime TaqMan Assay, PCR-RFLP, dHPLC).
MELAS mutation was found in 15 unrelated patients. Tests were conducted also in their family members, among whom another 13 cases of MELAS were found.
Molecular tests were performed on DNA samples isolated from blood leukocytes, buccal swabs, hair follicles, urine sediment cells and nails. In our group of MELAS patients A3243G mutation ratio is significantly higher in urine than in blood and other type of material. Measurement of A3243G mutation ratio in urine is a non-invasive, convenient and rapid method with its diagnostic meaning superior to blood testing. Direct sequencing might not be sufficiently sensitive method for detecting A3243G mutation.

P02.158
Hereditary palmoplantar keratoderma and hearing loss with mutations in GJB2 gene - A case report

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Mutations in the *GJB2* gene, encoding Connexin-26, are the most common cause of nonsydromic hearing loss with autosomal recessive inheritance (DFNB1), but also cause autosomal dominant hearing loss - isolated (DFNA3) or syndromic (KID, HID, Vohwinkel and Bart-Pumphrey syndromes).
We present a Czech family where 3 members suffer from hearing loss (HL) and palmoplantar keratoderma (PK).
The proband is a 22 year-old boy who presents late-onset epilepsy in addition to HL and PK. Since the first year of age the proband has shown several squamous focuses on his hands, feet, knees, and elbows. The skin lesions are progressive and have been becoming psoriasis-like and ichtyosis-like in character. The HL was verified at the age of 4, with a strong indication that it is congenital. The epilepsy appeared at the age of 12, presenting itself as motoric seizures during sleep, and is well balanced with Valproic Acid monotherapy. There is no pathology on MRI brain scan.
Mother and sister of the proband suffer from HL and PK, but not epilepsy. Proband's father is deaf after early meningitis and has no skin problems. All grandparents are hearing well and without any skin lesions.
We found two *GJB2* gene mutations in our patient - p.Arg75Trp (c.223C>T) and p.Met34Thr (c.101T>C). The first mutation has already been described as a pathogenic dominant mutation; however, the role of the second one still remains unclear. With regards to the low-compliance of the family, all molecular-genetic analysis are not yet completed.
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P02.159
Contributions about the incidence of hearing-speaking disorders in a population with mental deficiency

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Deafness is determined in many cases by genetic causes. For example, it is estimated that one of 1000 children has hereditary deafness. In 50% of cases, is a genetic cause, but is important to mention that in different families can occur diverse types of inheritance. We investigated 596 children interned in Neurology and Psychiatry Clinical Hospital of Oradea between 1999 and 2001 period. The methods utilised were cytogenetic, clinical investigations, somatometrical, statistical, psychiatric investigation and hearing tests, too. There were realised family investigations and were constructed pedigrees. We recorded 50 cases of hearing-speaking disorders in studied population, which means a frequency of 8,39%. Eight cases (16%) of all cases recorded, have no mental deficiency associated. Over 10% of the cases with mental deficiency have hearing-speaking disorders. It were observed increased frequencies in groups with moderate and severe mental deficiency. The frequency of hearing-speaking disorders in group with severe mental deficiency could be more increased because of the young age of children, when they can't cooperate with the person who investigate them. Most of cases proceed from rural area, perhaps because of agglomeration of some mutant genes. We could assume an autosomal recessive or dominant inheritance. Also, we could assume an X-linked inheritance.

P02.160
Study about the incidence of the congenital bony abnormalities in a population with mental deficiency

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It is known that genetic disorders cause mental disorders and malformative disorders, including bony abnormalities.The most frequent congenital bony disorders observed in the studied population are: congenital hip sprain, club foot, flat foot and vertebral column abnormalities. We investigated 596 children interned in Neurology and Psychiatry Clinical Hospital of Oradea between 1999 and 2001 period. In 596 children, 393 associated different levels of mental deficiency.

The methods utilised were cytogenetic, clinical, statistical, psychiatric investigation and were constructed pedigrees. We reported 81 cases of congenital bony disorders. In this total, 55 cases (67,9%) associated mental deficiency. In general, the frequency in rural has a meaningful increase than in urban. In studied population, 13 cases associated mild mental deficiency, 10 cases associated moderate level of mental retardation and 32 cases associated severe mental deficiency. Most of cases proceed from rural area. The most often is observed the congenital vertebral column disorders. The frequency is meaningfully increased in children group with severe mental deficiency. This result can be explained because of the existence of congenital bony abnormalities, such as common traits in chromosomal syndromes or other syndromes which cause plurimalformative phenotypes associated with mental deficiency.Cranial and facial dysmorphism has a frequency of 13%. This frequency is increased in the group of severe mental deficiency. These abnormalities could be caused by some genetic disorders which cause bony abnormalities, too. The explanation of these frequencies is the presence in the children group with associated mental deficiency of many genetic disorders which could cause congenital abnormalities.

P02.161
Deletion 10q24.32 in a patient presenting with a SMS-like phenotype and the undetectable level of L-DOPA in the cerebrospinal fluid

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aCGH has significantly improved the ability to identify cryptic chromosome rearrangements in patients with MR/MCA, dysmorphism, and behavioral abnormalities. We report on a 17-year-old patient with a Smith-Magenis syndrome-like phenotype, including mild intellectual impairment, hyperactivity, aggressiveness, and self-destructive behavior. Sleep disturbances and nocturnal cry have been noted from childhood. After an episode of seizures at 10 years, antiepileptic treatment was successfully initiated. No mutation or exonic deletion were found in RAI1. Using a clinical oligonucleotide aCGH, we have identified a unique ~ 317 kb hemizygous deletion at chromosome 10q24.32 harboring seven genes, including transcription factor PITX3 that is known to regulate the development of midbrain dopamine (DA) neurons together with other transcription factors. Pitx3-/- mice have a selective loss of dopaminergic neurons in the substantia nigra, leading to the significantly reduced DA levels in the nigrostriatal pathway and manifest anomalous striatum-dependent cognitive impairment and abnormal lens development. Heterozygous missense mutations in PITX3 have been reported in patients with dominant congenital cataract and anterior segment mesenchymal dysgenesis. Interestingly, no eye anomalies were found in our patient and analysis of neurotransmitters in his cerebrospinal fluid revealed undetectable level of L-DOPA and significantly decreased levels of catecholamine metabolites. Based on our data and the results of the experiments with dopamine agonists in the Pitx3 deficient mice, we suggest that the abnormal neurobehavioral phenotype in our patient, likely resulting from the decreased levels of dopamine, could be mitigated with tetrahydrobiopterin and/or L-DOPA.
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P02.162
Detection of deletions and duplications that cause mental retardation by whole genome array CGH

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Introduction: Mental retardation is one of the most common developmental disorder and its prevelance is known as %1-3 percentage of population. In the majority of mentally retarded individuals a specific cause can not be identified. Aim of this study was determining chromosomal aberrations in patients with mental retardation by scanning whole genome.

Materials and Methods: Peripheral blood samples were collected from 52 individuals between the ages of 0-18 with mental retardation regardless of gender. Genetic deletions/duplications were detected on DNA level using array-CGH method. Isolated gDNAs from peripheral blood samples were hybridized on CytoSure Syndrome Plus(v2) Microarray 4x44K microchips. Obtained data were analyzed using CytoSure Analysis Software v.2.0.8.

Results: Deletions and duplications with various sizes were detected in various regions of the genome on 38(73%) patients. No deletions/duplications were found on 14(27%) patients. Deletions/duplications with different sizes were found in different parts of the genome on 7(70%) of non-syndromic mental retardation patients, 22 (78.6%) of patients with mental retardation associated with dysmorphic findings, (87.5%) of patients with mental retardation accompanied by epilepsy and 2(33.3%) of patients with mental retardation associated with epilepsy and dysmorphism.

Conclusion: The diagnostic yield of array CGH in general population of individuals with mental retardation is at least twice as high as that of standart GTG-banded karyotyping and fluorescence in situ hybridization (FISH) in terms of high resolution. The limited ability to differentiate between inherited copy number variations (CNV) which cause abnormal phenotypes and rare variants unrelated to clinical alterations currently constitutes a limitation for guiding genetic counselling.

P02.163
Investigation of microdeletion syndromes by MLPA in Iranian patients with Mental Retardation

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Mental Retardation (MR), defined as a state of developmental deficit, results in significant limitation of intellect and poor adaptation behavior. Karyotyping is a usual method for primary screening of MR patients, however, detection of small duplications and deletions require a more sensitive technique. Because of the large number of known genes involved in MR, mutation screening in all of them is not a feasible and cost effective approach. Multiplex Ligation-dependent Probe Amplification (MLPA) has the ability of relative quantification of several target DNA sequence in a single reaction by using specific probes for each target.

During the last year a total of 71 patients with dysmorphism and MR have been referred to us. Initially, all patients were clinically evaluated and karyotype analyses were performed. All of the patients had normal karyotypes. By using MLPA method, we investigated duplications and deletions for 21 different microdeletion syndromes by MRC-Holland kit. We identified 10 (~14%) aberrations as follow (table):

Syndrome	Williams	Miller-Dieker	Sotos	DiGeorge	Angelman	Others
Chromosomal region	7q11.23/ del	17p13.3/ del	5q35.3 / del	22q11.21/ del	15q12/ del	4p16.3/ dup
No. of Probes	3	2	2	3	4	2
No. of patients detected	3	3	1	1	1	1

Our MLPA results indicate a high degree of concordance between the clinical data, and the genotype. We suggest MLPA as a first screening method for patients suffering from MR with normal karyotypes. In the case, in which clinical findings was not completely compatible with the microdeletion syndrome detected by MLPA, array CGH was performed revealing a larger duplication/deletion in the critical region.

Key words: Mental Retardation, MLPA, Iran

P02.164
Clinical features associated with submicroscopic chromosomal aberrations in patients with mental retardation/ developmental delay

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Objective: To compare the spectrum of congenital anomalies (CA) and minor anomalies (MA) between mentally retarded/developmentally delayed (MR/DD) patients with clinically significant CNVs and MR/DD patients with normal molecular karyotyping results.
Methods: Molecular karyotyping using oligo arrayCGH and SNP microarrays was performed on 114 MR/DD patients with/without CA and dysmorphic features. Based on the molecular karyotyping results, patients were divided into two groups. Group A consisted of patients with clinically significant CNVs (N=16) while group B consisted of patients with normal molecular karyotyping results (N=98). CA and MA were coded using Eurocat Malformation coding guides.
Results and Discussion: CNS congenital anomalies occurred more often in group A versus group B (31% versus 11%, *P*=0.048). No significant differences were found between these two groups when analyzing the rate of CA in the organ systems included in other blocks of the XVII chapter (ICD-10 classification, Q00-Q99). Interestingly, patients with chromosomal rearrangements (group A) were more likely to have at least one MA (*P*=0.02) and had a significantly higher frequency of eye, ear, face and neck MA (Q10-Q18) compared to patients from group B (68.8% versus 38.8%, *P*=0.04). The increased rate of CA in the CNS and MA of the eye, ear, face and neck in MR/DD patients with clinically significant CNVs provides useful information for counseling and may aid in the identification of submicroscopic chromosomal rearrangements in other patients.
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P02.165
Clinical and molecular characterization of two cases with rare chromosomal microduplications

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After introduction of methods for molecular karyotiping many new microdeletions/ microduplications were characterized and their association with specific phenotypes recognized. We report two patients with chromosome duplications involving regions associated with common microdeletion syndromes. Among 90 patients affected with mental retardation (MR) / developmental delay (DD) screened by MLPA for common microdeletion syndroms and subtelomeric rearrangements two were found to carry small duplications - at 3q29 and 10p15 respectively. Both patients are ascertained because of MR and dysmorphism of unknown etiology. Both cases are sporadic. At age of 13 years patient with 3q29 duplication presents mild MR, microcephaly and dysmorphism. Patient with duplication of 10p15 was refereed to our department at age of 18 months because of DD and epilepsy. After initial cytogenetic and metabolic investigations MLPA was performed using consecutively kits P245, P70 and P36 (MRC Holland). Duplications were detected with at least two of the used kits. Chromosomal rearrangements were further characterized using arrayCGH. Phenotypes of our patients share similarities with cases reported recently by other investigators.
Contiguous gene syndromes involving small chromosome microduplications are typically less frequently reported than their microdeletions counterparts. Although these rearrangements can arise from a common mechanism involving nonallelic homologous recombination, microduplication syndroms are usually less commonly recognized, possibly due to their milder and more variable phenotype and technical limitations of routine cytogenetics. Methods for molecular karyotiping are invaluable in clarifying the etiology of MR/DD and the approach of "reverse phenotypics" broadens our understanding of human genome architecture and functioning.

P02.166
Fryns mesomelic dysplasia: a new familial case

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Mesomelic dysplasias are a heterogeneous group of inherited skeletal dysplasias among which Leri-Weill dyschondrosteosis caused by SHOX haploinsufficiency and its homozygous form, Langer mesomelic dysplasia, are the most frequent and best known. In addition to Kantaputra dysplasia, an autosomal dominant form caused by duplications of the *HOXD* locus on chromosome 2q, various unclassified forms of unknown cause, mostly with a unique phenotype in single families, have been reported. In these observations, mesomelic dysplasia of the upper limbs was associated with involvement of the lower limbs and in most cases with other skeletal problems. However, in 1988, Fryns et *al.*, described a female patient and her father presenting with bilateral mesomelic dysplasia of the upper limbs without any other features. In 2005, Mégarbané et *al.* reported a similar clinical picture in a boy whose father, interestingly, had unilateral involvement. In both families, the patients had severe developmental abnormality of the ulna, which was extremely short and thick, associated with marked radial bowing, and resulting in a severely shortened and angulated forearm with ulnar deviation of the hand but with no Madelung deformity. The tibiae and fibulae were completely normal and the patients had normal stature.
We report a female patient and her similarly affected father who have exactly the same phenotype with mesomelic dysplasia confined to the upper limbs. This new family adds further arguments to the existence of a separate condition, probably autosomal dominant, consisting of mesomelic dysplasia of the upper limbs as the unique feature.

P02.167
New recessive syndrome of microcephaly, cerebellar hypoplasia, and congenital heart conduction defect

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We identified a two-branch consanguineous family in which 4 affected members (3 females and one male) presented with constitutive growth delay, severe psychomotor retardation, microcephaly, cerebellar hypoplasia, and second degree heart block. They also shared distinct facial features and similar appearance of their hands and feet. Childhood-onset insulin-dependent diabetes mellitus developed in one affected child around the age of 9. Molecular analysis excluded mutations in *PTF1A* locus and *EIF2AK3* gene being pathogenetics for insulin-dependent diabetes mellitus with cerebellar agenesis or microcephaly, respectively. To our knowledge, this phenotype does not meet diagnostic criteria of any known condition, and thus suggests a unique clinical entity of autosomal recessive mode of inheritance.
Key Words:
Microcephaly, insulin-dependent diabetes, cerebellar hypoplasia, mental retardation, heart block

P02.168
Septo-optic dysplasia: a new manifestation of microdeletion 22q11.2?

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22q11.2 microdeletion is a well known entity affecting potentially almost any organ and with more than 180 signs described. We report a female patient with a seemingly new manifestation, namely septo-optic dysplasia.

She was seen for genetic counseling at the age of 24 years because of mental handicap, short stature and visual impairment. Pregnancy and delivery were uneventful. Birth growth parameters were at P90. Nystagmus was present since birth and severe amblyopia diagnosed in infancy. Motor development was delayed, with walking at 5 years. Puberty was uneventful with menarche at 12 years and regular menstrual cycles. Complete growth hormone deficiency was diagnosed at 20 years and brain MRI showed septo-optic dysplasia with agenesis of the anterior pituitary. At examination, the patient was overanxious and talkative. Height was 139 cm (-4.4 SD), weight 44 kg (-1.5 SD) and OFC 52 cm (-2.5 SD). Erratic nystagmus, divergent left strabismus, broad neck and slender fingers were noted.

As the phenotype was not evocative of a known syndrome, array-CGH (Agilent 244k) was performed and showed, surprisingly, a classical 3 Mb *de novo* heterozygous 22q11.22 microdeletion, confirmed by FISH. To the best of our knowledge, septo-optic dysplasia has not been previously reported in microdeletion 22q11.2. Whether this is a new and rare finding associated with this deletion or the chance occurrence of two separate conditions (a frequent deletion on the one hand, and, on the other hand, a mutation in a gene such as *HESX1* causing in itself the dysplasia) cannot be answered at present.

P02.169

Subtelomeric study of the patients with developmental delay, dysmorphy and/or congenital anomalies of unexplained etiology

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Submicrosopic chromosomal rearrangements involving the subtelomeric regions are considered to be a significant cause of idiopathic mental retardations (MR). Their prevalence is about 5.2%, depending on the preselection criteria. Because of the small size of involved segments, these aberrations are undetectable by conventional banding techniques. Subtelomeric regions can be screened by multiplex ligation-dependent probe amplification (MLPA). We have studied a series of 60 unrelated patients with MR and normal results of GTG-banded chromosomes. Subtelomeric assays using MLPA with SALSA P036-E1 and P070-B1 kits were performed.

The MLPA screening revealed subtelomeric chromosome aberrations in three cases(5%).

Case 1: A 3- year-old girl, born from healthy unrelated parents, with growth retardation, facial dysmorphism, microcephaly, syndactily, tumor hepatis and severe mental retardation. Deletion of 1p and duplication 12q has been detected.

Case 2: A 28- month-old girl, born from healthy unrelated parents, with severe growth retardation of prenatal onset, mental retardation, mild facial dysmorphism, epilepsy and hemiparesis. Deletion of 4p has been detected.

Case 3: A 28- month-old girl, born from healthy unrelated parents, with craniostenosis, facial dysmorphism, microcephaly and moderate mental retardation. Deletion of 9p and duplication of 15q have been detected.

Parents of the patients with disclosed subtelomeric aberrations were also tested in order to clarify the origin of aberrations. In all cases de novo origin was confirmed.

These results demonstrated that MLPA may be very useful tool in order to approach genetic diagnosis of mentally retarded patients.

P02.170

Array-CGH (Agilent 244K and 4X140K) analysis in patients with MR/DD /MCA

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No of Pat.	Chromosome	Gain/ Loss	start/ end UCSC hg18	Length Range (Mb)
7	1p36.33-p36.32	4 L/ 3 G	554268->6141121	0.69->5.6
1	1p31.2-p22.3	1 L	69154817->87595434	18.44
1	1q21.1	1 G	145009467->146212234	1.2
1	1q44	1 L	242886313->244525547	1.64
1	2p16.3	1 L	51090504->51167934	0.077
2	2q24.2-q31.1	2 L	163364586->170720574	2.1->7.4
1	2q37.3	1 L	238465588->242690037	4.2
1	3p25.3	1 L	9499872->11406036	1.9
3	3p14.1	3 L	70598263->71601477	0.083->1.2
3	5p15.33	3 L	934536->2209449	1.01->1.26
4	5p15.2	4 L	11095452->11368310	0.034-
1	5q23.2-q31.1	1 L	124232611->135251538	>0.052
2	5q33.1	2 L	149716211 ->150082767	11 0.015->0.37
1	7p22.2-22.3	1 L	250232->2801328	2.55
3	7p22.2-p22.1	2 G; 1 L	4340544->7025368	1.7->2.7
2	7q11.23	2 L	72022051->73923281	0.029->1.9
5	8q24.23-q24.3	4 L, 1G	138221526->146250824	3.7-8
1	8q24.3	1 L	145944589->146264902	0,413
1	8q21.11-q21.13	1 L	77016764->80392452	3.4
1	9p24.3-p22.1	1 G	194193 ->19203881	11,0
1	9q31.2	1 L	107304497->109588754	2.3
4	9q34.2-34.3	4 L	134831651->140241935	0.7-5.4
1	13q33.1-q34	1 L	100510439 ->114114568	13.6
4	15q11.2-q14	2 L; 2 G	18362555->36837570	5->17.8
7	15q11.2	5 L; 2G	18683110->25373779	0.02->2.1
2	15q21.3-q22.31	2 L	54118678->62432440	8.13
5	16p13.3	4 L; 1 G	70350->3229290	0.08->2.9
1	16q21-q22.1	1 L	64772843->66806006	2.03
2	17q12	2 L	31474518->34217217	1.1->2.7
5	17q21.31-q21.32	3 L, 1 G	41288843->42142422	0.25->0.8
2	18p11.32-p11.21	2 L	4316->15370683	9.6->15.3
2	20q13.3	2 L	61433519->62419593	0.29->0.99
1	22q11.1-q11.21	1 G	15438946->17041773	1.6
1	22q11.21-q11.23	1 L	20128705->21984222	1.86
1	22q11.21	1 L	17299942->19794119	2.5
1	Xp22.32	1 G	5818688->5918660	0.1
3	Xp22.31	3 G	6477006->8124803	1.6
1	Xp11.3	1 L	43208140->43765770	0.56
1	Xp11.22	1 L	53238335->53251848	0.013
2	Xq13.3	2 L	74410307->74516096	0.041->0.1
2	Xq28	2 L	151370278->153127086	1.5
1	Xq28	1 L	153248667-> 153335811	0.087

Clinical characteristics of patients are not always related to specific syndromes. Array comparative genomic hybridisation (aCGH) is used to detect small copy number changes within the genome that are not always visible by conventional karyotyping. We analysed with aCGH (Agilent 244K & 4x140K), 253 patients with various degrees of MR, DD, dysmorphic features and/or single or multiple congenital abnormalities, normal previous conventional karyotype and negative genetic tests (FRAX, RETT, single FISH or metabolic screens). Clinically significant submicroscopic imbalances were detected in 92 (~30%) patients. The high percentage of positive patients is probably due to the strict criteria of patient selection. Some patients presented with more than one aberration relevant to their phenotype. A total of eleven aberrations were detected on the X chromosome regarding

genes related to MR/ DD. Array CGH has proven a powerful tool for the identification of novel chromosomal syndromes, for more accurate prognosis and phenotype-genotype correlations.

P02.171
A novel deletion at 3q13.31-22.1 causes dysmorphia and multiple congenital malformations.

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We describe a 14-year-old Polish girl with dysmorphic features and multiple congenital malformations, who carries de novo heterozygous deletion of 15Mb at 3q13.31-22. The child's clinical presentation included mental retardation, epilepsy, myopia, hypothyroidism, and hypotonia. The dysmorphic features included: macrostomia, narrow left palpebral fissure, right auricular tags, short stature, scoliosis, contraction of the left forearm and left hand phalangeal hypoplasia, absent radial bones, hypoplastic thumbs, left clubfoot varus, and prominent clitoris. Moreover, the child demonstrated the following congenital malformations: right renal agenesis, grade IV left vesicoureteral reflux, left thyroid lobe aplasia, patent ductus arteriosus, and hypoplasia of corpus callosum. The karyotype was 46XX. We performed a genome-wide analysis of copy number variants using the Illumina 610-Quad array. We identified a large deletion of 14,960,554 bp at the 3q13.31-22.1 locus that was absent in public databases and >15,000 controls. The deletion was confirmed by qPCR and SNP genotyping, and was absent in both parents, indicating a de novo event. These data provide strong support for pathogenicity.

P02.172
Autosomal dominant natal teeth with selective tooth agenesis

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We report a 5-generation family with multiple natal teeth and selective tooth agenesis segregating in an autosomal dominant fashion. Natal teeth are usually a sporadic isolated finding in an otherwise normal infant, and familial occurrence is uncommon. Selective tooth agenesis is usually genetic and not associated with natal teeth. In affected persons in the family we report, at least 6-8 natal teeth are usually present at birth, and this is followed by selective tooth agenesis that results in absence of as many as 16 permanent teeth. Natal teeth with hypodontia occurs in some ectodermal dysplasia syndromes. In the family we report, natal teeth with selective tooth agenesis did not include ectodermal dysplasia or any other problem. DNA from 28 family members was analyzed on the Illumina OMNI-express chip using 733,120 SNPs and mapped to an approximately 2Mb segment on chromosome 1q36.11 with LOD score 2.97 at 23.8 Mb to 25.8 MB (GRCh37/hg19; MERLIN). By dividing the pedigree into three 3-generation families, a region of association was found located between LOC284632 and GRHL3 (parenTDT, p=0.005 for rs11249039, rs11249045, or rs7526505). GRHL3 is a gene expressed exclusively in surface ectoderm in drosophila, where it plays an essential role in cuticle formation. Expression of the murine Grhl3 gene is evident in ectodermally derived tissues, including the oral epithelium. We speculate that variation in the regulation of this gene may play a role in the phenotype we describe in this family.

P02.173
Nemaline myopathy caused by mutations in the nebulin gene may present as a distal myopathy

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Congenital myopathies are a group of disorders characterised by muscle weakness due to abnormalities in muscle fibres, disturbing their normal function. The muscle biopsy usually also shows abnormal structures, such as protein aggregates. Mutations in the nebulin gene (*NEB*) are the main cause of autosomal recessive nemaline myopathy (NM), with clinical presentations ranging from mild to severe proximal muscle weakness and protein aggregates, nemaline bodies, seen in the muscle fibres. At present over 130 NM-causing *NEB* mutations have been identified in more than 110 families. In addition to mutations causing NM, compound heterozygous mutations in *NEB* have been identified causing core-rod myopathy (CRM) in one patient, and homozygous missense mutations in *NEB* causing distal myopathy with no or almost no nemaline bodies in four different Finnish families. It was concluded that perhaps the presence of two missense mutations in *NEB* would lead to distal myopathy, while more disruptive compound heterozygous mutations would cause NM or CRM. Recently, however, we have identified four different compound heterozygous *NEB* mutations, only one of which is a missense mutation, in three non-Finnish patients in two unrelated families with distal myopathy and nemaline bodies in their muscle biopies. One of the mutations has previously been identified in one family with the severe form of NM. We conclude that NM and distal myopathy caused by *NEB* mutations form a clinical and histological continuum. NM should be considered as a differential diagnosis in patients presenting with an early-onset, predominantly distal myopathy.

P02.174
Molecular investigation of neonatal hypotonia : 27 floppy infants

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The neonatal hypotonia is the call sign of many diseases of the newborn and infant. Taking into account the diseases covered by a molecular diagnosis certainty and with neonatal hypotonia as a major clinical sign, we are interested in three neurological disorders: Prader Willi Syndrome (PWS), spinal muscular atrophy type I (AMSI), and myotonic dystrophy type 1 (DM1).

The aim of our work is to establish a molecular diagnosis strategy of neonatal hypotonia with neurological origin (SPW, AMSI, and DM1).

Our study was performed in 27 newborns referred to our laboratory for neonatal hypotonia. We carried the diagnosis of PWS by both MS-MLPA techniques and TP-PCR, for AMSI we have deployed the technique of PCR-RFLP, for the DM1, both techniques of TP-PCR and QMPSF were used. Patients without clinical suspicion of predilection were investigated with all these techniques.

The PWS diagnosis was confirmed in only one patient. Among 17 patients with AMSI suspicion this diagnosis was confirmed for only 7. For the remaining 19 patients none of the 3 molecular diagnostics (AMSI, DM1 and SPW) could be confirmed and etiology of neonatal hypotonia is to unveil.

Our work provides clinicians a strategy for the diagnosis of neonatal hypotonia. However this strategy remains incomplete and a multitude of other etiologies will have to be explored.

P02.175
Nijmegen breakage syndrome in siblings with atypical cardiac involvement

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Background: Nijmegen breakage syndrome (NBS) is a rare autosomal recessive congenital disorder, caused by a mutation in the *NBS* gene, located on chromosome 8q21.

Aim: To present two siblings with NBS, a boy 8 yrs old and a girl 3

yrs old, detected because of progressive microcephaly and recurrent sino-pulmonary infections. The boy was born with a severe cyanotic congenital heart defect (CHD).
Material and methods: We performed clinical, cardiology, neurology, ultrasound and genetic examination on both patients, followed by complex laboratory investigations.
Results: The patients have a distinctive facial appearance, microcephaly, short stature, recurrent infections and multiple splenic tumours. The sister also has convergent strabismus of the right eye, right palpebral ptosis, areas of skin hypopigmentation, and severe IgA immunodeficiency. She was treated with intravenous immunoglobulin. The boy has CHD with double outlet right ventricle, ventricular septal defect, inefficient pulmonary artery banding, severe pulmonary artery hypertension, NYHA III cardiac failure, cachexia, severe thrombocytopenia, severe motor delay, low mental retardation, and cellular immunodeficiency. A paravertebral T8-T9 tumour was detected in the boy. He was treated for heart failure. Thrombocytopenia is a contraindication for treatment of pulmonary arterial hypertension. Splenic tumours and paravetebral tumour have to be investigated. Genetic tests are in progress for both, looking for mutation, and colony survival assay. Parents have Slavic origin.
Conclusions: NBS is rare in siblings and needs to be diagnosed because of immunodeficiency that has to be treated, because of radiation sensitivity, which means radiation exposure must be avoided, and a strong predisposition to lymphoid malignancy, which has to be sought. CHD is a particular association.

P02.176
NIPBL gene mutation analyses among 70 Polish CdLS patients

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Cornelia de Lange Syndrome (CdLS) (MIM 122470) is a rare dominantly inherited multisystem disorder, characterised by a typical but variable phenotype, which includes developmental delay, characteristic facial features, pre- and post-natal growth retardation and limb abnormalities. About 50-60% of cases of CdLS are caused by mutations in one of three genes, mainly in a regulator of cohesion - NIPBL, and less frequently in two building the cohesin ring - SMC1 and SMC3. Cohesin is involved in control chromosome segregation during cell divisions and also in double strand break repair and/or transcriptional regulation. We screened 32 patients with the CdLS classical phenotype and 38 with mild phenotype for NIPBL point mutations, using a combination of denaturing high-performance liquid chromatography and direct sequencing. We identified 26 different NIPBL sequence variants. Eight of them were frame shift (exclusively present in the classical type), 15 were missense and three splice mutations resulting in exon skipping. Moreover, within the group of patients without identifiable point mutations, we performed an MLPA analysis and detected two heterozygous deletions of the entire NIPBL gene, confirmed by aCGH. In our group, NIPBL point mutations account for 37% of CdLS patients. Large rearrangements were detected only twice, which explains only 7% of all detected mutations. These findings are in agreement with other published data.

P02.177
The phenotypic variability in Noonan syndrome - Clinical consideration on two new cases diagnosed in neonatal period

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Noonan Syndrome (NS) is a common autosomal dominant disorder that affects both sexes. The main features are congenital heart defect, short stature, learning problems, pectus excavatum, impaired blood clotting, webbed neck, and a facial dysmorphism. The incidence is 1/1,000- 1/2,500 newborn. The range and severity of features can vary greatly in patients with NS, and thus the diagnosis is often delayed. We present two cases of NS in newborns with clinical features that attest the phenotypic variability of disease. Case 1: girl, born at term, APGAR 7, first child of an apparently healthy couple; clinical examination: dysmorphic face with: hypertelorism, down-slanting eyes, small and upturned nose, low set and backward rotated ears, short and webbed neck, muscular hypotonia, without any type of cardiac problems. The clinical examination of the mother indicated a phenotype of NS with short stature, mild mental retardation, short and webbed neck, facial dysmorphism, without cardiac problems. Case 2: girl, born premature at 35 weeks, APGAR 5, after a pregnancy complicated with polyhidramnios detected at 22 weeks, 2nd children of a healthy couple; clinical examination: generalized lymphoedema, dysmorphic face with hypertelorism, small and upturned nose, short palpebral fissures, short and webbed neck, thoracic hypoplasia, and cardiac anomalies: pulmonary valvular stenosis, and ventricular septal defect, hepatomegalia. Unfortunately, the girl dies at 2 days after delivery. In both cases the karyotype was normal: 46,XX and we have not had the opportunity for molecular diagnosis. Our cases confirm the phenotypic variability in NS and the importance of clinical examination.

P02.178
A patient with hyperactivity, speech delay and dysmorphic features associated with a deletion of the *NRXN1* gene at 2p16.3 and a 10q22.3q23.1 duplication

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We report on a 7 years old boy with hyperactivity, concentration difficulties, speech delay and discrete dysmorphic features. The patient is the third child to non-consanguineous parents. A sister is healthy and a twin brother has suspected attention deficit disorder (ADD). The mother has a family history of ADD, autism spectrum disorder and mild mental retardation.
Micro-array analysis of the proband revealed a 174 kb deletion at 2p16.3 inherited from the healthy father and a 508 kb duplication at 10q22.3q23.1 inherited from the mother. The deletion comprises the *NRXN1* gene but no other coding sequences. *NRXN1* has previously been associated with autism spectrum disorders and the deletion can, in part, explain the neuropsychiatric features of the boy. Furthermore *NRXN1* gene variants may segregate with a reduced penetrance[1] explaining the inheritance from a healthy father. The maternal family history and the complex phenotype in the boy, including dysmorphisms, made us also consider the maternally inherited duplication. The duplication spans 5 genes; *ANXA1, CTSLL6, MAT1A, PLAC9* and *SFTPD*. The gene product of *ANXA1* has been implemented as a central player in the anti-inflammatory and neuroprotective role of microglia[2]. *MAT1A* is the only gene that is sensitive to haplo-insufficiency and MAT deficiency can lead to neurological abnormalities, including brain demyelination[3]. Studies are ongoing to further investigate the phenotype of the proband and the genotypes of additional family members.

P02.179
A 10.46 Mb 12p11.1-12.1 interstitial deletion coincident with a 0.19 Mb *NRXN1* deletion detected by array CGH in a girl with scoliosis and autism

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We present a 12-year-old girl with de novo karyotype 46,XX,del(12)(p11.1p12.1). Array CGH revealed in addition to a 10.466 Mb interstitial deletion on 12p11.1→12p12.1 a 0.191 Mb deletion on 2p16.3. The girl presented with mild facial dysmorphism consisting of microcephaly, hypertelorism, downslanting palpebral fissures, strabismus, broad nasal base, bulbous nose, short philtrum, micro/retrognathia, irregular tooth arrangement, phalangeal deformity in distal phalanges of hands, 5th finger camptodactyly, brachydactyly in feet, history of joint hypermobility and scoliosis. She was considered to have mild to moderate mental retardation and ascertained for an autism spectrum disorder. Interstitial deletions of short arm of chromosome 12 are rarely reported whereas point mutations and deletions of *NRXN1* which is located on chromosome 2p16.3 are associated with autism spectrum

disorders. In this article we present and discuss the phenotypic consequences of a patient who was effected by deletions of two different chromosomal regions.

P02.180
Three uncommon cases of OAVS

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Oculo-auriculo-vertebral spectrum (OAVS) is a heterogeneous condition results from a defect of blastogenesis involving abnormal development of facial structures derived from the first and second branchial arches with in same cases cardiac, vertebral and CNS defects.

We describe three uncommon newborns' case with OAVS. First is a male with OAVS and severe caudal regression: the clinical features were marked right hemifacial microsomia, hypoplasia of the right mandibular ramus and right external ear with atresic external auditory canal and ossicles agenesia; "frog-like" appearance of the lower extremities, flexed knees due to bilateral popliteal pterigium and equinovarus deformity of bilateral feet. Abdomen US showed crossed renal ectopia, lower spine X-rays revealed absence of vertebra below D12 level, spine and brain RMI showed the spinal cord terminated at D6 level and corpus callosum hypoplasia. The association between OAVS and caudal regression is a typical manifestations of the axial mesodermal dysplasia complex.

The second is a female with OAVS and left labial cleft: the clinical features were left hemifacial microsomia, microtia, left labial cleft and dysmorphic right ear implanted in mandibular region.

The third is a female with OAVS and anterior cervical meningocele: the clinical features were left hemifacial microsomia, hypoplasia of the left mandibular ramus and reduced motility of the tongue due to a functional deficit of hypoglossal nerve and anterior cervical meningocele.

So the management of OAVS requires a multidisciplinary approach to provide the most appropriate treatment even more if, as in our cases, typical manifestations are associated with other specific malformations.

P02.181
Association of mitochondrial disorders with oxidative stress in obsessive compulsive disorder

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Obsessive compulsive disorder (OCD) is a common psychiatric disorder defined by the presence of obsessive thoughts and repetitive compulsive actions. The mutations or polymorphic variants in mitochondrial DNA-encoded genes or nuclear genes result in oxidative stress, which has recently been associated with various psychiatric disorders. In order to understand the association of mitochondrial disorders with oxidative stress in OCD, we examined genetic variants of MnSOD and UCP-2 antioxidant genes, whose product imported into mitochondrion, and MDA and GSH, markers of oxidative stress. The study sample comprised 102 patients with OCD and 104 healthy controls. For MnSOD, the frequencies of CT (Ala/Val) genotype ($p<0.01$) in patients were significantly lower than those of controls. However, CC (Ala/Ala) genotype was significantly more frequent in patients than controls ($p<0.05$). For UCP-2 (I/D), the frequencies of ID genotype ($p<0.01$) and I allele ($p<0.05$) were lower in patients as compared with controls. In contrast, DD genotype was more prevalent in patients than controls ($p<0.01$). While serum GSH was significantly depleted ($p<0.0001$), serum MDA was significantly elevated in patients compared with controls ($p<0.0001$). MDA levels were significantly elevated in subjects with DD genotype of UCP-2 (I/D) ($p<0.05$) and CC genotype of MnSOD ($p<0.05$) as compared with II or ID and TT or CT genotype, respectively. MDA levels in patients carrying CC ($p<0.05$) or CT ($p<0.05$) genotype were significantly higher than those of carrying TT genotype. In conclusion, CC genotype of MnSOD or DD genotype of UCP-2 might result in mitochondrial disorders by increasing oxidative stress in OCD.

P02.182
Oculoauriculovertebral spectrum: 1.34 Mb duplication in 14q23.1 in a family with autosomal dominant inheritance

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Introduction: Oculoauriculo vertebral spectrum or OAVS (OMIM 164210) is a clinically and genetically heterogeneous congenital disorder involving developmental defects of first and second branchial arch derivatives. Main features are unilateral/bilateral ear anomalies (anotia, microtia, preauricular tags, pits), hemifacial microsomia, ocular defects and vertebral malformations. Other features are cleft lip and/or palate, cardiac, cerebral , renal malformations and mental retardation. Diverse chromosomal abnormalities have been associated with OAVS (trisomies/monosomies).

Clinical Report: We report a 31 year old patient, with clinical diagnosis of Treacher Collins syndrome and several affected family members suggestive of autosomal dominant inheritance. Normal pregnancy and delivery. No gestational diabetes, uterine malformations, toxic environment. Physical examination: Long narrow face, bilateral preauricular pits and tags, left anotia, agenesis of middle and inner left ear, micrognatia, macrostomia, high arched palate. Length: 167 cm, PC: 55 cm. Normal cardiological evaluation. Spine Xray: dorsolumbar scoliosis. No vertebral defects. Father: right macrostomia. No hearing defect. Born with preauricular tags. Other 4 family members with preauricular tags, and macrostomia; suggestive of autosomal dominant inheritance. High density array-CGH was performed: 1,34 Mb duplication at 14q23.1.

Discussion: This is the second report of a 14q23 duplication in OAVS with autosomal dominant inheritance (previous description with a 11.79 Mb duplication at 14q22.3-23.3 with 4.38 Mb deletion at 13q21.31-q21.32: complex rearrangement). We discuss clinical heterogeneity as well as support the fact that 14q23 is a candidate region for OAVS phenotype. Our case narrows down the candidate region to 1,34 Mb, including SIX1 and SIX6 genes.

P02.183
Novel pathogen mutation in TYR gene in an Iranian patient with Oculocutaneous Albinism Type I(OCAI)

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Oculocutaneous albinism (OCA) is a severe genetic disorder characterized by reduced or absent biosynthesis of melanin pigment in melanocytes of the skin, hair follicle, and eye. Type I OCA results from deficient activity of melanocyte tyrosinase, a copper containing enzyme that catalyzes the first two steps in the melanin biosynthetic pathway. In classic, type IA OCA tyrosinase activity and melanin biosynthesis are entirely absent, and in type IB OCA tyrosinase activity and melanin production are greatly reduced. Types IA and IB OCA were result from allelic mutations of the tyrosinase gene.

In This study a patient with clinical phenotype of OCA I and her parents were investigated for mutations in TYR gene by PCR and sequencing method. This patient was homozygote for c: 89 T → A mutation that changes amino acid Cysteine to Serine while her parents were heterozygous for this mutation. This mutation was not reported previously and was not found in healthy controls and literatures. We believed that this mutation may have effect in patient phenotype.

P02.184
Oesophageal atresia with tracheoesophageal fistula, anal atresia and thumbs hypoplasia in a patient with a de novo gain in 17q12 detected by genome wide SNP array analysis.

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Oesophageal atresia (OA) and tracheoesophageal fistula (TOF) are foregut malformations with a heterogeneous etiology. OA/TOF may occur as an isolated anomaly or as part of a syndrome. Several

syndromes have OA/TOF as a clinical feature. The best known is the VACTERL association that is seen in 10-30% of OA/TOF. Chromosomal anomalies have been reported in 6-10% of OA/TOF, but no single specific chromosomal defect has been confirmed as a main etiological factor. Several genes have been implicated in cases of syndromic OA/TOF. It is thought that a combination of genetic and environmental factors play a role in the etiology of OA/TOF.
We report a male infant, born at 33 weeks of gestation with IUGR presenting the combination of multiple congenital defects (OA/TOF, anal atresia, thumbs hypoplasia, tracheomalacia, sacral bone defect, cryptorchidism) resembling VATER association. Follow-up examination showed psychomotor and somatic retardation. Genotype analysis revealed a normal male karyotype and absence of subtelomeric imbalances. Genome wide 250k SNP array analysis showed a 1.4Mb gain in 17q12. This gain appeared to have occurred de novo (normal result in the patient's parents) and is likely to explain the clinical picture of this patient.
Recently, aberrations of this 17q12 region that is flanked by low-copy repeats have been described by Nagamani et al. in patients with renal defects, epilepsy, brain abnormalities and intellectual disability. Among these patients, one presented with OA/TOF and vertebral defect.
We propose to consider a role for selected genes in the 17q12 region in the development of OA/TOF.

P02.185
GLI3 is rarely implicated in OFD syndrome with midline abnormalities

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Oral-facial-digital (OFD) syndromes represent a heterogeneous group of clinical entity characterized by the association of malformations of the face, oral cavity and extremities. Thirteen subtypes have been currently distinguished, characterized by specific extra-OFD features. Only the *OFD1* gene has been identified in the OFD type I and mutations in the *CORS2* gene in two patients with an OFD type VI. Recently, 6 mutations within the *GLI3* gene, responsible for Greig cephalopolysyndactyly and Pallister-Hall syndrome (PHS), have been reported in some cases (6/21 cases, 29%) who presented features of OFD syndrome associated to midline abnormalities. Five of the 6 mutations were similar in position within *GLI3* to other mutations that have been described in PHS. We report the results of *GLI3* sequencing analysis in 8 cases with OFD syndrome and midline abnormalities, including pituitary gland deficit (5/8 cases) with pituitary stalk interruption (3/5 cases), hypothalamic hamartoma (HH) (1/8 cases), corpus callosum agenesis (1/8 cases) and imperforate anus (1/8 cases). No *GLI3* mutation was identified. The difference between our cohort and the previous published results could be explained by the frequent HH (4/6 cases) in the previous published data and the rare prevalence (1/8 cases) in our cohort. When pooling with the previous studies, the frequency of *GLI3* mutations in OFD syndromes with midline abnormalities is 21%, and 36% if a HH is associated. In conclusion, further studies are required in order to evaluate the implication of the *GLI3* gene in OFD phenotype with midline abnormalities.

P02.186
A novel mutation in SALL4 causes Okihiro Syndrome with mainly skeletal involvement

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Okihiro syndrome (Duane-Radial Ray syndrome; DRRS) is an autosomal dominant condition characterized by upper limbs anomalies (radial ray defects), improper eye movements (Duane anomaly), and congenital bilateral non-progressive sensorineural and/or conductive hearing loss. Other less consistent deformities include anal, renal, cardiac, ear, and foot malformations. This disease results from of a mutation in the SALL4 gene, a human gene related to the developmental regulator spalt (sal) of Drosophila melanogaster. SALL4 mutations may also cause Acro-Renal-Ocular syndrome (AROS), which is characterized by radial ray malformations, renal abnormalities, ocular coloboma, and Duane anomaly. The majority of the mutations are truncating point mutations, which are expected to result in nonsense-mediated mRNA decay and are considered to cause the phenotype via haploinsufficiency. The only truncating mutation predicted to escape nonsense-mediated mRNA decay is associated with extensive clinical variability and severe hemifacial microsomia in one member of a large family.The only missense mutation identified so far is predicted to cause an increase of DNA binding capacity and is associated with central midline defects [Kolhase J, GeneReviews 2008]. Here we present an Austrian family with four members affected by Okihiro syndrome, in which we have identified a novel SALL4 mutation in exon 2 leading to an in frame deletion **c.694_696delACC (p.T232del)**. Family members had varying degrees of radial ray malformation and facial characteristics without Duane anomaly, but no hearing loss, nor malformation of other organs. This is the first report of an in frame deletion of SALL4 that causes Okihiro syndrome.

P02.187
A diagnosis of X-linked Opitz G/BBB syndrome by array-CGH in a boy and a girl

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X-linked Opitz G/BBB syndrome (OS) is characterized by facial dysmorphisms, laryngo-tracheo-esophageal and genitourinary abnormalities with mental retardation in 50% of (male) patients. *MID1* analysis detects mutations, deletions or insertions in 15-45% of males clinically diagnosed with OS. We report a male and female patient in whom the diagnosis of OS was either confirmed or suggested by array-CGH.
A girl with severe mental retardation had facial dysmorphisms including hypertelorism. Additional abnormalities included laryngotracheomalacia, tracheo-esophageal fistula, and absent puberty. Sequencing of *MID1* revealed no mutations. However, array-CGH showed a deletion in Xp22.2p22.32, including *MID1*, as well as *KAL1*, *STS* and *OA1*. X-inactivation studies are pending. Female carriers of a *MID1* mutation usually manifest only hypertelorism. A few male cases with a deletion of the entire *MID1*-gene have been reported, but to our knowledge this is the first female patient with a *MID1* deletion.
A boy presented with cryptorchidism, kidney cysts, glottic web, heart defect and thin corpus callosum. Dysmorphic features included hypertelorism, dysplastic ears and a midline defect of his upper lip. Array-CGH revealed two duplications in Xp22.2, with one of the breakpoints mapping within the *MID1*-gene and therefore possibly disrupting it. Mate-pair analysis could provide insight into the structure of *MID1* in this patient. One patient with OS due to a duplication of the first exon of *MID1* has been reported in the literature.
These cases illustrate that there can be a role for array-CGH in the diagnosis of OS and show an example of full OS in a female patient.

P02.188
Large duplication of the MID1 gene in a patient with Opitz GBBB syndrome

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Opitz GBBB syndrome (OS, MIM145410 and MIM300000) is a congenital midline malformation syndrome characterized by hypertelorism, hypospadias, cleft lip/palate, laryngotracheoesophageal abnormalities, imperforate anus and developmental delay. The X-linked form is caused by a large variety of mutations in the MID1 gene. They involve point mutations, small sized (less than 1kb) insertions, deletions and duplications.
Here we report a maternally inherited duplication involving part of MID1 gene, minimum 37kb in size, in a 2-months-old-boy with Opitz

syndrome. The patient presented with feeding and swallowing difficulties and following features: brachycephaly, large fontanel, pronounced hypertelorism, shallow orbits, alternated divergent strabismus, broad flat base of the nose, mid-cleft of the tongue, short fraenulum, high palate and posteriorly rotated ears. Laryngooesophagofibroscopy and X-ray failed to detect laryngotracheoesophageal abnormalities. SNPs array analysis (Affymetrix 250k NspI) detected a 37-176kb duplication which involves exon 1 of MID1 gene (NM_000381.2) and spans between base pair X:10415285-10451959 (NCBI36/hg 18). The aberration and its maternal origin were confirmed by MLPA. Patients' mother showed subtle clinical features, such as feeding difficulties in the infancy, hypertelorism and shallow orbits. Variable clinical features of OS presented also maternal grandfather, his sister and her son. All affected family members have normal intelligence.
This report defines a novel type of genetic defect in patients with X-linked OS that is undetectable by the classical sequence analysis on the DNA level. Further studies for larger duplications/deletions of the MID1 gene on patients with a clinical diagnosis Opitz syndrome may be appropriate and could confirm the molecular diagnosis.

P02.189
De novo R304W mutation in the P63 gene associated with variable expressivity of the EEC syndrome

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Mutations in the transcription factor gene p63 is associated with several syndromes each including various combinations of limb malformations ectodermal dysplasia and orofacial clefting. Ectrodactyly, ectodermal, clefting (EEC, OMIM 604292) is the prototype of these syndromes. Most patients present with all three components.
We present a case with mutation in p63 and no limb involvement The proband is a 7 years old boy who was born at term to unrelated parents of Jewish origin. He had cleft lip and palate which as well as lacrimal duct stenosis which were corrected. He has spars thin and pale hair. He has hypodontia with 17 teeth missing. On his back there are hyperpigmented lesions. No abnormality was noted in his hands feet or nails. He has no abnormal sweating, no hearing impairment or kidney abnormality. He has no developmental problems.
A diagnosis of EEC syndrome was suspected. Screening for mutations in the p63 gene revealed an R304W mutation, known to be a disease causing mutation in this syndrome. The parents do not carry this mutation.
Even though ectrodactyly is a hallmark in EEC syndrome and is found in the vast majority of the patients, it should be included in the differential diagnosis when the other two major components exist (i.e. ectodermal and clefting). Lack of involvement of the limbs in this boy may be due to other genes affecting the expression of the mutated p63 in this case pointing at a more complex mechanism for the mild phenotype in this boy.

P02.190
A boy with intranasal hamartomatous polyp and pericallosal lipoma further expands Pai syndrome phenotype.

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Pai syndrome is characterized by mild hypertelorism, midline cleft lip, nasal and facial polyps, pericallosal lipoma, ocular anomalies and normal neuropsychologic development.
We describe an 8 years old child presenting with congenital nasal hamartomatous polyp and peri-callosal lipoma. The association of these two extremely rare findings in the same patient is highly suggestive of Pai syndrome. Thirty patients with Pai syndrome are described in the literature. Nasal polyp is a constant feature in Pai syndrome patients described so far. Peri-callosal lipoma was present in all but one patients screened with brain MRI. Another common feature is midline cleft lip described in 26/30 patients. In view of this case and literature review, we suggest as minimal clinical criteria for Pai syndrome: congenital midline nasal polyp, peri-callosal lipoma and/or midline cleft lip. Furthermore, our patient expands the phenotype of Pai syndrome with some atypical findings such as bucco-lingual dyspraxia, true tracheal bronchus, sacral dimple and hypospadias.

P02.191
Study of allelic variants in patients with neuropathic pain from Salamanca (Spain)

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Introduction
Perception of pain is a complex process which implies multiple biochemical pathways together with unknown processes of cortical integration. The existence of individual differences in the response to painful stimuli suggest that genetic factors can be involved in its modulation. The aim of our study was to investigate the genetic variation of 25 genes involved in different pathways of pain in patients from Salamanca (Spain), diagnosed with neuropathic pain.
Methods
Genomic DNA was extracted from peripheral blood leukocytes by standard techniques. We selected 29 non-synonymus SNPs. Studies were performed using TaqMan probes (Applied biosystems) for the analysis of the polimorphisms in the following genes: IL6, IL4, IL1B, NOS, eNOS, nNOS, TRPV, GSTM1, GSTT1, GSTP1, CYP2D6, COMT, PTGS2, HTR2A, SLLC6A4, OPRD, OPRM, OPRK, CNR1, DRD2, GABRA1, GABRA6, PPARG, EDN1, BDNF and CHRNA5.
Statistical analysis was performed comparing the different allelic variants of the genes in subgruopus of patients: patients with a VAS below and over 50.
Results and conclusion
Preliminary analysis has shown significant differences ($p<0.05$) comparing both groups of patients in the IL1B and PTGS2 genes. Il1B is an important mediator of the inflammatory response, involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. PTGS2 is the key enzyme in biosynthesis of the prostanoids, regulated by specific stimulatory events, suggesting that it is responsible for the prostanoid biosynthesis involved in inflammation and mitogenesis.
This results support the hypothesis that polymorphisms in genes involved in different pathways could be asociated with increased susceptibility to suffer pain.

P02.192
INew mutation of ARX gene as a cause of Partington syndrome.

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Infantile spasms, mental retardation, autism, and dystonia represent disabling diseases for which little etiologic information is available. Mutations in the Aristaless related homeobox gene (ARX) have been found in patients with these conditions .Aristaless related homeobox (ARX) is a transcription factor containing highly conserved octapeptide, homeobox, acidic, and aristaless domains, as well as four polyA tracts.. The most common mutation, the c.428_451dup, is associated with a wide spectrum of phenotypes, ranging from the most severe West syndrome to Partington syndrome (MR and hand dystonia), and even nonsyndromic X-linked mental retardation (NS-XLMR). Here, we report the boy with clinical manifestation of Partington syndrome (PS) , where the mutation of poly A tract is slightly different: c.426_458 dup. The boy presents with brachial dystonia, ataxia and severe mental retardation- typical symptomps of PS. .
Mutations in the homeobox gene, ARX, cause a diverse spectrum of disease that includes cognitive impairment, epilepsy, and in another group of patients severe cortical malformations. Although the precise prevalence of ARX mutations is unclear, ARX may rival Fragile X as a cause of mental retardation and epilepsy in males.
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P02.193
Bilateral persistent hyperplastic primary vitreous - case report

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Background: Persistent hyperplastic primary vitreous (PHPV) is a rare congenital condition due to failure of regression of the embryological, primary vitreous and hyaloid vasculature. It typically presents unilaterally (90%) without associated systemic findings. Between the three variants of PHPV(anterior, posterior or a combination of anterior and posterior presentations) the last one is the most commonly seen. The patient usually has microophthalmia, leukocoria and reduced vision. Cataract and anomalies of the anterior chamber angle are present. Accompanying retinal lesions (detachment, dysplasia) may be seen. Other complications in PHPV are : amblyopia; strabismus; loss of vision; glaucoma.The treatment is surgical and the procedures depend on the clinical presentation. Early surgical rehabilitation may facilitate a better visual outcome. Material and methods: We present a 3 years old girl with visual impairment and moderate psychomotor delay. She is the product of an uncomplicated full-term pregnancy. In her mother's family are present some cases of cataract and strabismus. Full evaluation (history, clinical exam, biological and imagistic tests, ophthalmological, neurological and genetic evaluation, audiometry) was done. Results: Bilateral strabismus, enophtalmia, amblyopia and cataract were noticed. Ocular ultrasonography revealed bilateral persistent hyperplastic primary vitreous. No hearing impairment was identified. Patient's family disagrees with surgical eye procedures (vitrectomy). Conclusions: The visual prognosis is poor in this case because it is a bilateral complicated presentation of PHPV. The delay of the surgical rehabilitation would lead to irreversible amblyopia, even loss of vision. Genetic and family counseling, ophthalmological and neurological follow up are mandatory.

P02.194

Variation of clinical expression in family with Pfeiffer syndrome caused by p.P252R mutation in FGFR1 gene

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Pfeiffer syndrome (OMIM#101600) is a genetic disorder which belongs to craniofacial dysostosis group, inherited autosomal dominant with change in clinical expression. It affects about 1 in 100,000 live-births. Typical features include: premature fusion of certain bones of the skull (craniosynostosis), maxillary hypoplasia and digital abnormalities of the hands and feet. This condition can be caused by heterozygous mutations in either fibroblast growth factor receptor gene type 1 or 2 (*FGFR1* or *FGFR2*). *FGFR1* mutations often result in less severe craniofacial involvement and abnormalities of the limbs.
The family of three members (father, his son and daughter) affected by Pfeiffer syndrome has been reported. Father demonstrated brachycephaly, hypertelorism, midfacial hypoplasia, stiff and broad thumbs, 2rd and 3rd toe syndactyly, very broad, flat, medially deviated great toes and normally developed intelligence. Son during examination showed severe mental retardation without ability of speaking, total 2rd to 4rd syndactyly toes at both feet, slightly medially deviated great toes and he did not express craniosynostosis features. In case of daughter insignificant signs of stiff thumbs, slight, partial 2rd and 3rd toes syndactyly without craniosynostosis features and normal intelligence were reported. In affected family we showed p.P252R mutation in *FGFR1* gene.

P02.195

Pitt Hopkins syndrome: further delineation of the neurological phenotype

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Pitt Hopkins syndrome (PHS, MIM#610954) patients present with severe intellectual disability with no speech, typical facial gestalt and hyperventilation episodes. In 2007, TCF4 was indentified as the disease causing gene with de novo heterozygous mutations or deletions. Since, about 60 patients have been reported.
Here, we report a novel series of 30 patients diagnosed with PHS and refine the neurological and morphological phenotype.
Within a two years period we identified heterozygous de novo TCF4 gene mutations or deletions in 30 patients with severe intellectual disability and the PHS facial gestalt. All patients were clinically revaluated with special attention to neurological presentation, with films whenever possible, and available brain-MRIs were reviewed.
All our patients had severe intellectual disabilities with no speech. Twenty-seven have facial features typical for the syndrome. A majority of the patients presents stereotypic movements (arm flapping, rapid movement of fingers, hand nibbling or rubbing and head rotation) and restless movements increasing with anxiety and excitation. Hyperventilation is frequent and often triggered by anxiety or excitement. Epilepsy and microcephaly are very inconstant findings. Cerebral MRI shows minor morphological changes only.
PHS is not rare among patients with severe intellectual deficiency. We report the clinical features of 30 PHS patients, with special emphasis on the neurological phenotype and key diagnostic features in the Rett-like group of patients. Genotype-phenotype correlation will also be discussed.

P02.196

Mutation analysis of the NPHS2 gene with the direct DNA sequencing method in Russian children with steroid-resistant nephritic syndrome.

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The NPHS2 gene encodes podocin protein, which plays an important role in glomerular ultrafiltration and controlling slit membrane permeability. Podocin is a raft associated component of the glomerular membrane where it is localized at the insertion of the slit-diaphragm. It was known that mutations of an NPHS2 can determine specificity of treatment for children with nephrotic syndrome (NS). In Russian patients with steroid-resistant nephrotic syndrome (SRNS) mutations of the NPHS2 gene have remained mainly unknown. The aim of present study was to screen for podocin mutations in Russian children with SRNS. There were 61 patients with SRNS and 15 age-matched healthy children. Mean age at onset of proteinuria was 6,3±4,8 years. Mutation analysis was performed in eight exons of the NPHS2 gene using the direct DNA sequencing method. We identified mutations and polymorphism variants in the NPHS2 gene: rs3738423 (S96S), rs61747728 (R229Q), rs1410592 (A318A), rs3818587 (L346L), rs1410592 (A318A), IVS3+1507 C>T, IVS3+1460 C>T, IVS7+7 A>G, IVS7+1078 G>C. First discovered in the Russian patients the

polymorphism IVS7+7 A>G (N.Poltavetz) was also represented in our SRNS patients. We revealed two new mutations I255T and V338L. It is known that R229Q polymorphism associates with the SRNS in different ethnic groups. In our study frequency of R229Q polymorphism in children with SRNS have met in two times often than in the healthy group. The frequencies of other polymorphisms was approximately the same in both groups. Thus the detected NPHS2 gene polymorphisms in our patients must be borne in mind when choosing therapy of SRNS.

P02.197
Two cases of Pontocerebellar Hypoplasia Type 2: clinical, neuroradiological and molecular genetical findings

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Pontocerebellar hypoplasias (PCH) are rare autosomal recessive neurodegenerative disorders which are characterised by severe mental and motor impairments, hypoplasia of the cerebellum and ventral pons, microcephaly, and variable degrees of cortical atrophy. PCH are subdivided into six types (PCH1 to PCH6), of which PCH2 is most common accounting for about 90% of all PCH cases.

PCH2 is characterised by progressive microcephaly from birth combined with extrapyramidal dyskinesia, lack of voluntary motor or mental development, severe chorea, and frequently epilepsy. It is caused by biallelic mutations in *TSEN54*, whereby a homozygous c.919G>T (p.Ala307Ser) mutation has been identified in approximately 90% of PCH2 cases. This mutation is thought to be a founder mutation descending of a single couple that lived in the seventeenth century in Volendam, The Netherlands. Most children die before age ten years, although survival beyond age 20 has been reported. Typical findings in PCH2 in cerebral Magnetic Resonance Imaging (cMRI) are, beyond ventral pontine atrophy/hypotrophy, cerebellar hypoplasia whereby the cerebellar hemispheres are more affected than cerebellar vermis with a relative sparing of the flocculi. These cMRI features were termed as dragonfly-like cerebellar pattern and recognised as pathognomic for *TSEN54* mutations.

Here we report on two female patients aged 9 months and 13 years, respectively. Both children were born by non consanguineous parents of German descent. After an uneventful pregnancy and birth, they developed neurological signs resembling to PCH. Cranial MRI displayed typical pattern of PCH2. Sequencing of *TSEN54* revealed homozygousity for the *TSEN54* c.919G>T mutation in both patients.

P02.198
Psychological and behavioral profile of Romanian Prader Willi patients

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Prader Willi syndrome (PWS) is a complex disorder that includes prenatal/neonatal central hypotonia, poor suck and infantile failure-to-thrive, characteristic facial appearance,

developmental delay, childhood onset obesity, short stature, hypogonadism and a characteristic behavior disorder.

We have studied the 57 patients registered with Romanian Prader Willi Association in order to appreciate the psychological and behavioral profile. The major aims of this study were to identify specific behavioral patterns and early ways to improve developmental delay and behavioral problems, for the benefit of patient and family. Criteria studied included concordance of the different developmental fields (motor/language/cognitive), severity of developmental delay, personality (stubbornness, preferences, communication, manipulation, selfmutilation etc) and evolution of personality characteristics in time, memory and functioning, sleep problems and how do they influence behavior, evolution of hypotonia in time. After a global analysis, the cases have been grouped according to the genetic defect and all these parameters have been compared.

Statistics for all these criteria will be provided, as well as a comprehensive model for the investigation, education and therapy offered to a child diagnosed with PWS, in order to reduce major psychological and behavioral problems. Particularities found will be underlined.

In conclusion, we have analyzed the psychological and behavioral profile of 57 cases diagnosed with PWS. Based on this, we have elaborated a model of investigation, education and therapy in order to optimize the recovery of the psychological problems that these patients are facing.

P02.199
Prader-Willi/ Angelman syndromes: different molecular approaches.

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Prader-Willi (PWS) and Angelman (AS) syndromes are distinct neurogenetic disorders, caused by chromosomal deletions, uniparental disomy or loss of the imprinted gene expression in 15q11-13 region. While PWS occurs from a lack of the paternally expressed gene contribution, AS originates from a lack of the maternally expressed gene expression in the region, specifically *UBE3A*.

The aim of the study was to evaluate the clinical diagnosis using different comparative molecular methods.

The study group consisted in 14 children (age 2-10 years) presumptively diagnosed with PWS and one case with AS at UMF Timisoara- Pediatric Hospital and 10 normal patients (control).

DNA and RNA samples were isolated from white cell blood. For MS-PCR 700ng of DNA was sodium bisufite treated. RNA samples were reverstranscribed with Access Quick Kit (Promega). Epigenetic changes at SNRPN gene locus were evaluated with MS-PCR technique. Two non-imprinted genes expression (NIPA1 and OCA2) was evaluated by qReal-Time PCR for identification of deletions type 1,2,3. SALSA MS-MLPA kit ME028 was used to detect copy number changes and to analyze CpG islands methylation of the 15q11 region. 4/14 children presented deletions type 1,2,3 and 6/14 display imprinting defect (all PWS). In children with 15q11-13 deletions, no or poor NIPA1, OCA2 gene expression was found, depending on deletion type. The deletions was confirmed in FISH and MLPA analysis thus recommending NIPA1 and OCA2 gene expression as an alternate method for deletions investigation.

P02.200
The oro-maxillo-facial anomalies in patients with Prader Willi syndrome: a survey of 13 cases

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Prader Willi syndrome PWS is a chromosomal microdeletion or disomy disorder due to deletion or maternal disomy 15q11-13. It is characterized by severe neonatal hypotonia, childhood-onset hyperphagia and obesity, short stature, facial dysmorphy (narrow bifrontal diameter, almond-shaped palpebral fissures, down turned mouth), hypogonadism, mental retardation and anomalies of the oral region.

The purpose of this report is to evaluate carefully the patients with PWS and to describe the oro-maxillo-facial anomalies.

We followed 13 PWS patients (age range 26 years- 5 years 8 months; 8 girls, 4 boys) in Centres for Medical Genetics Iasi and Timisoara: dysmorphologic and clinical examination, dietetic and GH treatment history, cognitive function and behavior problems, oral and radiologic exam, reviewed all the laboratory studies.

All the patients presented characteristic facial features, thick saliva, speech articulation defects, 2 patients had microstoma, 1 with microdontia, 5 with malloclusion, 5 with enamel dysplasia, and 9 patients presented multiple dental caries (erosive and pour oral hygiene, bruxism).

PWS is frequent associated with oro-maxillo-facial anomalies and typically dysmorphic face. Clinical proper management is essential for a favorable oral health status.

P02.201
Early death in an infant diagnosed with Prader-Willi syndrome

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Prader-Willi syndrome (PWS), a disorder with severe implications in child pathology marked by hypotonia in neonate period and obesity after the age of two years may have fatal evolution. The death causes may vary according to patient's age and also depend of the initiation of growth hormone therapy. We report the history of a PWS case with early death. The male infant delivered by caesarian section with low birth parameters, due to breech presentation, presented hypotonia, but no feeding difficulties. The clinical examination revealed: high forehead, slight upslanting palpebral fissures, low nasal bridge, microretrognathia, short neck and cryptorchidia. Conventional cytogenetic analysis was performed and the karyotype was normal. Due to hypotonia hormonal profile was evaluated: FT3, FT4, and TSH indicating congenital hypothyroidism defined later as transitory. At age of 7 months the persistent hypotonia, imposed the microdeletion FISH analysis with SNRPN probe for the Prader-Willy syndrome, which confirmed the diagnosis. Echocardiography revealed hypoxic hypertrophic cardiomyopathy with atrial septal defect. In evolution metabolic disturbances were identified with high levels of cholesterol, glycemia and GPT. The investigation of gonadotrophins sexual hormones indicated high levels of LH and low levels of FSH and testosterone. The patient was admitted several times due to recurrent episodes de bronchiolitis and pneumonia and at the age of eight months died due to cardio respiratory failure. For the next pregnancy the parents agreed to prenatal diagnosis although the recurrence risk is only 1-2% due microdeletion etiology of the case.

P02.202
Diagnosis in Prader-Willi Syndrome by methylation test in Brazil

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PWS is caused by loss of paternal contribution of genes located on chromosome 15 (15q11-13). Most of patients show obesity and mental disability. Holms *et al. (1993)* proposed the criteria for its clinical diagnosis. These criteria were revised by Gunay-Aygun *et al*. (2001) aiming to define criteria for selecting patients who should undergo molecular testing. The aim of this work was to identify PWS in 34 Brazilian patients with putative diagnosis, and to verify whether the molecular results were compatible with the clinical diagnosis based on Holms and Gunay-Aygun's criteria. The patients were sent to the Genetics Outpatient Clinic of HUGG by different clinicians. They were reassessed and had the methylation test done. The method employed McrBC endonuclease that digests the site 5'...Pu^{m}C(N40-3000) Pu^{m}C...3' of exon-1 of *SNRPN* gene (methylated only in normal chromosomes of maternal origin). Amplification of exon-1 *SNRPN* gene was achieved by PCR with *SNRPN* primers. The amplicons were detected by 3% agarose gel if the SPW region of paternal origin was normal. This method can detect SPW caused by deletion or abnormal methylation on chromosome 15 of paternal origin, as well as by maternal uniparental disomy. Out of 34 patients, 23 were judged clinically by Holms criteria and 29 were offered the molecular test based on Gunay-Aygun. We found 8 patients with a molecular result compatible with PWS. Therefore, PWS is best diagnosed clinically by experienced clinicians backed by DNA analysis. The diagnosis should be done as early as possible for appropriate counselling and dietary management initiation.

P02.203
Estimation of prevalence of malformation syndrome by population-based birth defects monitoring system in Japan

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Multiple malformation syndromes are recognized by characteristic anomalies and need specialized medical cares. For the study of genetic diseases, epidemiology plays a central role in public health, improvement, and disease prevention. However, evaluation and ascertainment of the incidence of these disorders is limited because of difficulties of diagnosis and rarity in general population. To elucidate the incidence of malformation syndromes, we used data from population-based monitoring system and genetic clinic of highly specialized children's hospital in the same prefecture. KAMP is one of the population-based birth defects monitoring program operating from 1981 in Kanagawa Prefecture, Japan. It covers a half of total births, 40,000 annually. During the study period, the annual number of patients with Down syndrome in the genetic clinic has been correlated with the prevalence in the KAMP. These results suggested the hypothesis that other recognizable malformation syndromes may visit the clinic with the same ratio. According to the known prevalence of Down syndrome (1/800), we calculated the incidence of the recognizable malformation syndromes. The estimation indicated that the incidence of Costello syndrome is 1/60,000-120,000 births, CHARGE syndrome 1/30,000 births, Rubinstein-Taybi syndrome 1/50,000-60,000 births, Young-Simpson syndrome 1/100,000 births, and ATR-X syndrome 1/60,000 male births respectively. Although the syndromes are very rare, the total number of diseases is high and has large impacts on the public health. This methodology may be successfully applied to other malformation syndromes.

P02.204
Dopamine gene receptors expression in psoriatic patients

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Dopamine plays roles as a neurotransmitter and neuromodulator with functions in brain and peripheral tissues. Dopamine receptor (DRD) subtypes are related to depression and also have been shown to be present in certain cells of the immune system. There are some evidences that suggest that DRD subtypes are responsible for high lymphocyte activation in psoriasis. Psoriasis is a proliferative and inflammatory disease. There are at least five subtypes of DRDs, D1, D2, D3, D4, and D5. The D1 and D5 receptors are members of the D1-like family of DRDs, whereas the D2, D3 and D4 receptors are members of the D2-like family. The aim of this study is evaluation of DRD (3,5) genes expression in psoriatic patients in comparison with normal individuals by Real Time PCR.
The PBMC was separated from whole blood by Ficoll-hypaque. The total cellular RNA was extracted and the cDNA was synthesized. This process was followed by real-time PCR using primer pairs specific for DRD mRNA and beta-actin as internal control. Results show relative overexpression of DRD3 gene has significant changes between two groups, but we could not find significant changes between two groups for DRD5 gene. Considering these results the present study has shown a change in DRD3 gene expression in PBMC of psoriasis patients. Due to this data, we conclude that significant increased expression of DRD3 gene may has an important role in psoriasis.

P02.205
PTEN-Associated Macrocephaly/Autism Syndrome

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There is a strong genetic component to autism spectrum disorders (ASDs) but due to significant genetic heterogeneity individual genetic abnormalities contribute a small percentage to overall total.
PTEN hamartoma tumour syndrome (PHTS) is a heterogeneous group of disorders (Cowden syndrome, Bannayan-Riley-Ruvalcaba

syndrome, Proteus syndrome and Proteus-like syndrome) characterized by germline mutations in the PTEN gene and an increased risk of different tumours.
Previous studies have proved PTEN mutations in a portion of individuals with ASDs and macrocephaly that do not exhibit features of PHTS.
From a group of patients currently genetically investigated in our institute we have selected 21 children with a head circumference range from 2.0 to 4.8 standard deviations above the mean for the PTEN mutation analysis. Three novel (p.Asp331ThrfsX11, p.Thr321GlnfsX23, p.Glu242X) and one known germline mutation (p.Pro246Leu) have been found in four (19%) of 21 probands.
Our data support former findings that PTEN mutations are relatively frequent in children with ASDs and macrocephaly and therefore PTEN gene testing should be considered in such patients. The gene findings may impact on assessment of the recurrence risk as well as medical management of early cancer prevention.
The work was supported by the grant MZOFNM2005 and CHERISH

P02.206
Absence of influence of gender and BMPR2 mutation type on clinical phenotypes of pulmonary arterial hypertension

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Background: Previous studies indicate that patients with pulmonary arterial hypertension (PAH) carrying a mutation in the bone morphogenetic protein receptor type 2 (BMPR2) gene, develop the disease 10 years earlier than non-carriers, and have a more severe hemodynamic compromise at diagnosis.
Methods: We reviewed data from all patients with PAH considered as idiopathic and patients with a family history of PAH who underwent genetic counseling. We compared clinical, functional, and hemodynamic characteristics between carriers and non-carriers of a BMPR2 mutation, according to gender or BMPR2 mutation type.
Results: PAH patients carrying a BMPR2 mutation (n=115) were significantly younger at diagnosis than non-carriers (n=267) (35.8±15.4 and 47.5±16.2 respectively, p<0.0001). The presence of a BMPR2 mutation was associated with a younger age at diagnosis in females (36.4±14.9 in BMPR2 mutation carriers and 47.4±15.8 in non-carriers, p<0.0001), and males (34.6±16.8 in BMPR2 mutation carriers and 47.8±17.1 in non-carriers, p<0.0001). BMPR2 mutation carriers had a more severe hemodynamic compromise at diagnosis, but this was not influenced by gender. No differences in survival and time to death or lung transplantation were found in male and female PAH patients carrying a BMPR2 mutation. No differences were observed in clinical outcomes according to the type of BMPR2 mutations (missense, truncating, large rearrangement or splice defect).
Conclusion: When compared to non-carriers, BMPR2 mutation carriers from the French PAH network are younger at diagnosis and present with a more severe hemodynamic compromise, irrespective of gender. Moreover, BMPR2 mutation type had no influence on clinical phenotypes in our patient population.

P02.207
UPK3A and FGF7 mutation analysis in Dutch renal adysplasia patients provides further evidence for the role of UPK3A in congenital anomalies of the kidneys and urinary tract (CAKUT) pathogenesis

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Renal adysplasia is part of the spectrum of congenital anomalies of the kidney and urinary tract (CAKUT) that forms a major cause of end-stage renal disease in children. Little is known about the origin of renal dysplasia, though it is anticipated that genetic and environmental factors are involved. There is a role for genes expressed during early nephrogenesis in CAKUT etiology. In this study, two genes, uroplakin 3A (UPK3A) and fibroblast growth factor 7 (FGF7), were screened for variants in a phenotypically diverse cohort of 19 Dutch renal adysplasia patients. Four novel, inherited, UPK3A mutations were identified in 3/19 (16%) patients with unilateral multicystic dysplastic kidney. The mutations - c.356T>C (p.Ile119Thr), c.418G>A (p.Gly140Arg), c.450C>A (p.Gly150Gly) and c.545G>A (p.Trp182X) - were not described before and not observed in 96 control chromosomes. As c.418G>A was detected in a patient with VACTERL association (Vertebral defects, Anal atresia or stenosis, Cardiac defects, Tracheo-Esophageal fistula, Radial defects and Renal anomalies, Limb defects), 25 additional DNA samples of VACTERL cases were screened; no mutations in UPK3A were detected. In FGF7, no likely pathogenic mutations were detected. This is the first time a stop-mutation in UPK3A is reported. All UPK3A mutations published so far were reviewed and in silico analyses are presented. This study revealed novel UPK3A mutations strengthening the position of variants in UPK3A in the etiology of renal adysplasia.

P02.208
The low penetrance of retinoblastoma for the V654L mutation in RB1 gene

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Background
Retinoblastoma is caused by compound heterozygosity or homozygosity of retinoblastoma gene (*RB1*) mutations. In germlinal retinoblastoma, mutations in *RB1* gene predispose increase cancer risk during development and segregates as an autosomal dominant trait with high penetrance (90%).
Methods
In this study, we screened 30 family members from a certain families using high resolution melting assay (HRM) and DNA direct sequencing. We evaluate the phenotype and penetrance of a germ-line mutation of *RB1 gene* in a large Taiwanese family.
Results
The molecular analysis and clinical details of this family showed phenotypic variability associated with certain V654L mutation in exon 19 of *RB1* gene in 11 individuals. The phenotype varies from asymptomatic to unilateral tumor. Only 4 individuals (2 males and 2 females) developed unilateral retinoblastoma, which results in calculated low penetrance of 36% (4/11). In particular, none of them relatives in this family exhibited the variable severity and bilateral retinoblastoma.
ConclusionsThe diseased-eye ratio (DER) for this family was 0.36, lower than current estimates. This suggests that the *RB1* V654L mutation was itself insufficient to develop the retinoblastoma.

P02.209
A case of bilateral retinoblastoma with later development of a jaw osteosarcoma as a second primary malignant tumor

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Introduction
Retinoblastoma represents a rare type of eye malignant tumor that usually develops in early childhood in the retina. Most cases present inactivating mutations in the RB1 gene, located on chromosome 13q14, a tumor suppressor gene which regulates the cell growth. 40 % of all retinoblastomas are germinal, concerning all bilateral forms and 15% of the unilateral ones. The mutation in the RB1 gene is inherited in a autosomal dominant pattern, with a high penetrance rate. Patients

with this form have an increased risk of developing second site primary malignant tumors, including osteosarcoma , pinealoblastoma, Ewing sarcoma, leukaemia, lymphoma, epithelial or brain tumors. Genetic counseling and analysis are indicated for every patient, including an anticipation examination schedule for testing and direct and indirect molecular tests.

Case presentation

A 17 years old female presented with a large, unilateral tumor located on the right upper jaw. From her antecedents we mention: she was born at full term, by normal vaginal delivery, she had developmental milestones adequate for the age. At the age of three she was operated and then chemo and radio treated for bilateral retinoblastoma. Family history for retinoblastoma was negative (including a brother and a sister). The upper maxilla tumor was histopathologically diagnosed as a osteosarcoma.

Conclusion

The patient presented bilateral retinoblastoma in childhood, caused by germline mutation of the RB1 gene, surgical, chemo and radio treated, with an increased risk of developing second site primary malignant tumors, which explains the later development of a jaw osteosarcoma in her case.

P02.210
Novel triple deletion of the MECP2 gene in atypical Rett syndrome patient

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Rett syndrome (RTT) is an X-linked dominant neurodevelopmental disorder with variable clinical presentation. About 75-90% patients with classical and 40% with variant RTT have heterozygous mutation in the X-linked MECP2 gene. We report on a 5.5 year old girl with a novel C-terminal MECP2 triple deletion and atypical clinical presentation. The girl was born at term after an uneventful pregnancy. Her development was slow from birth. She started sitting at the age of 12 months and walking at the age of 18 months. By the age of 1 year she acquired several words, thereafter speech deteriorated. Postnatal deceleration of head growth was not present. She was evaluated at the age of 2 years and 8 months due to moderate global developmental delay and behavioural problems (aggressiveness, hyperactivity, screaming and laughing spells and impaired social interaction). At that time her height was 95.5cm (+1SD), weight 18.5kg (+2.5SD), head circumference 50cm (+1SD). On follow up no progression of mental deterioration was observed. Her gait is only mildly disturbed, she is able to walk unsupported, purposeful hand use is preserved, with only occasional stereotypic movements. DNA bidirectional sequencing of MECP2 coding exons revealed de novo triple deletion in C-terminal region of MECP2 gene - c. [1021_1037del17; 1057_1089del33; 1162_1179del18,] not previously described. Result was confirmed with MLPA analysis. The variability of the clinical presentation of RTT emphasizes the need of the molecular analysis of MECP2 gene in all girls with developmental delay.

P02.211
The age related changes of phenotype in boy with ring chromosome 15 and del(15)(q26.2-qter) together with dup(15)(q26.1) .

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In case of ring chromosomes occurring in karyotype the monosomy of the terminal long arm of ring chromosome and mitotic ring instability are taking into consideration as a explanation of peculiar morphologic and behavioral phenotype. Recently inv dup del rearrangements have been described in some of ring chromosomes which may influenced phenotype. We present natural history of 26-years old of boy with developmental delay, short stature and peculiar morphological phenotype and late onset diabetes mellitus type 2. The ring chromosome 15 in his karyotype were detected using GTG and RBG banding techniques. The loss of chromosome material from short and long arm of chromosome 15 leading to monosomy 15q26.2→qter were confirmed by FISH using IGF1R probe (Posejdon). The analysis performed by array-CGH technique with resolution of about 60 kb (kit 105K AGILENT) has revealed: a duplication of about 6.7 Mb of the long arm of a chromosome 15 [dup(15)(q26.1)], with proximal breakpoint falling between 88,860 Mb (normal) end 88,885 Mb (duplicated) and a deletion of about 4.8 Mb of the long arm of a chromosome 15 [del(15)(q26.2→qter)] with proximal breakpoint between 88,885 Mb (duplicated) and 95,606 Mb (deleted). Phenotype -genotype correlation changing with the age was evaluated using dysmorphological protocol of Stengel-Rutkowski et al for quantitative phenotype evaluation of boy for each from 4 periods of development separately and differences have been observed.

P02.212
Rubinstein-Taybi syndrome: case report

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Rubinstein-Taybi syndrome (RSTS; MIM180849) is a congenital disease characterized by postnatal growth deficiency, microcephaly, specific facial characteristics, broad thumbs and big toes, and mental retardation. Estimated prevalence of RSTS is 1 per 10,000 live births. It occurs generally sporadic, and can be caused by a microdeletion of chromosome 16p13.3, or by mutations in CREB or EP300 genes. Here we reported on 6 years old girl with multiple congenital anomalies and mental retardation. Proband was born premature at 35-36 weeks of seventh pregnancy complicated with anemia, threatened miscarriages at 10-12, 29-30 and 34-35 weeks of gestation. She was the second child of non-consanguineous parents. At birth her father was 25 years aged. Her mother 27 years aged suffered from thyroid enlargement, chronic urogenital infection and had a professional contact with industrial hazards. Proband's birth weight was 1950g and length was 42 cm. At 6 yr her weight was 10,000g, her length 68cm, chest circumference 51cm, head circumference 49cm. Clinical findings of the proband 6 yr aged included mental retardation and speech difficulties, facial abnormalities (down-slanting palpebral fissures, highly arched eyebrows, ptosis, epicanthal folds, strabismus, OD with atypical cataract, hypermetropia of high degree, astigmatism, OS with hypermetropia of average degree and astigmatism, prominent beaked nose, hypoplastic maxilla with narrow palate, malformed ears), digit abnormalities (broad great toes and thumbs, broadness of other fingers, persistent fetal finger pads, syndactyly), cardiac symptoms (tricuspid regurgitation, incomplete right bundle-branch block, premature ventricular repolarization). X-ray examination of upper extremity showed marked malformation of hand bones.

P02.213
Clinical and molecular analyses in a series of 28 patients carrying a pathogenic *SALL4* mutation

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Introduction. We report on a series of 28 patients, 16 probands and 12 relatives, presenting with heterozygous *SALL4* mutations on chromosome 20q13.13-q13.2.

Patients. Sixteen probands were initially referred for Okihiro syndrome

(7/16), or other overlapping phenotypes such as Holt-Oram (8/16) or Townes-Brocks (1/16) syndromes. After clinical expertise, final considered diagnoses were Okihiro (11) and Holt-Oram (5) syndromes.
Methods. The clinical and molecular characteristics were analyzed in order to identify genotype-phenotype correlations. Clinical details were obtained from the referent practitioners. Direct sequencing of the complete *SALL4* coding region and quantitative real time PCR were performed. After identification of the causal mutation or deletion, 12 affected relatives were also tested.
Results. We identified 100% radial ray malformations (28/28), 39% Duane anomalies (11/28), 28.5% renal anomalies (8/28), 25% heart defects (7/28), 21% growth-retardation (6/28), 14% deafness (4/28), 11% scoliosis (3/28) and 11% gastrointestinal abnormalities (3/28). Most of the patients carry either a frameshift mutation or a deletion in *SALL4* within exons 2 (68%: 19/28) and 3 (21%: 6/28). Two patients were mutated in exon 4, and 1 presented a complete *SALL4* deletion. Thirteen mutations had never been reported previously.
Discussion. We confirm in this large series that *SALL4* mutations cause a range of variable phenotypes, including Okihiro and Holt-Oram syndromes. These results suggest that all patients presenting with a radial ray malformation should be referred to an ophthalmologist and tested for *SALL4* if an anomaly was to be identified. The clinical characteristics are more precisely defined but genotype-phenotype correlations are not yet obvious.

P02.214
Role of the SCN1A gene in the pathogenesis of familiar febrile seizures and GEFS+

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Febrile seizures (FS) are epileptic phenomena occurring in course of fever in children with an age ranging from 6 months to 6 years old. FS are the most common type of epilepsy in childhood, with an incidence rate of 2-14%. If febrile seizures persist after six years old, they are considered as an epileptic syndrome named GEFS+, a rare familiar disease that arises as FS and progresses to generalized epilepsy in adults. GEFS+ presents an extremely heterogeneous spectrum of epileptic phenotypes whose acuteness varies from mild to severe.
It is well known that the gene coding the α-1 sodium channel subunit (SCN1A) is mutated in GEFS+ patients; moreover it has been recently suggested as a possible susceptibility locus for FS. We investigated the involvement of SCN1A in a group of GEFS+ and FS families by means of mutation analysis. The study indentified two missense mutations (R542Q and T297I) in highly conserved regions of the channel together with several known SNPs. The R542Q is a de novo mutation already known to be associated with both autism and myoclonic epilepsy, while the T297I, previously associated with SMEI, was identified in all affected members of a family showing FS.
Functional studies aimed at evaluating the effect of these mutations on the physiological properties of the channel are currently in progress. Preliminary results suggest that these variations can compromise the functionality of the protein altering the activation/inactivation properties of the channel.

P02.215
New genetic variation (Y87C) in SCN5A gene in patient with asymptomatic Brugada syndrome

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Background: Mutations in the cardiac sodium channel gene (SCN5A) have been identified in people with Brugada syndrome, which causes idiopathic ventricular fibrillation and sudden cardiac death (SCD).
Methods: We have screened coding and contiguous intronic areas of SCN5A gene by DNA sequencing in asymptomatic patient, who was examined in our Centre. He has been observed by physical examination, standard ECG, 24-h Holter monitoring and Echo-CG.
Results: Asymptomatic male patient (46 y.o.) with spontaneous Brugada syndrome type 1 characterized ECG-pattern BrS 1 revealed during planned examination. There were no syncopes, ventricular fibrillation episode, but his son (23 y.o.) also had ECG-pattern BrS 1. There were no observations of sudden cardiac death and heart diseases in this family.
A single nucleotide substitution of A to G at nucleotide position 260 changed the coding sense of exon 2 of the SCN5A from tyrosine to cysteine (Y87C) was found.
Conclusion: We recently identified the Y87C variation by DNA sequencing of SCN5A, which have not been previously described.
A new variation may be associated with the risk of sudden cardiac death. Population analysis of ethnically-matched controls will be perfomed.
This work was partly supported by grant N°02740110783.

P02.216
Screening of BSCL2 in Spanish patients with spastic paraparesis and motor neuropathies.

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BACKGROUND: While recessive mutations in Seipin/BSCL2 cause Berardinelli-Seip lipodystrophy, dominant gain-of-toxic-function mutations affect upper and lower motor neurons, causing Silver syndrome (SS), hereditary spastic paraplegia (HSP-SPG17), distal hereditary motor neuropathy (dHMN) and Charcot-Marie-Tooth disease type 2 (CMT2). p.N88S and p.S90L in the glycosylation site are the only mutations identified in neurologic seipinopathies, however screening was generally limited to the canonical 398 aa seipin isoform. A longer isoform of 462 aa has been proposed but its role remains unknown.
AIMS: To investigate the contribution of BSCL2 to HSP and motor neuropathies.
METHODS: Sequencing the whole coding region of the large BSCL2 isoform (NM_001130702.1; NP_001124174.1) in: a) 106 cases of spastic paraplegia without mutations in spastin and atlastin; b) 10 patients with peripheral neuropathy and pyramidal signs without mutations in other CMT genes. Sequence variations were checked in 180 control individuals.
RESULTS: One family with the p.N152S (p.N88S in short isoform) mutation showed manifestation spectrum from pure-HSP to SS and pyramidal signs without subjective symptoms. One family with the p.S154L (p.S90L) mutation presented pure HSP, CMT2 or dHMN. Seven new BSCL2 sequence variations were detected (4 missense, one in-frame deletion, two intronic).
CONCLUSIONS: Seipin mutations account for at least 1% of spastic paraparesis and 10% of motor neuropathies with pyramidal signs in our series. Variable expression within a given kindred is the rule, difficulting diagnostic awareness. The role of BSCL2 isoforms in the nervous system, as well as the functional effect of sequence variants in motor neuron disorders remains to be elucidated.

P02.217
Scalp-Ear-Nipple (Finlay-Marks) syndrome. A new case with renal involvement.Thirty six year follow-up and molecular cytogenetic studies.

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Finlay-Marks syndrome, or Scalp-Ear-Nipple (SEN) syndrome (MIM ID %181270) is a very rare Autosomal Dominant disorder, first described in 1978 by Finlay and Marks in a family with 10 affected individuals over five generations. The clinical diagnosis is based on the occurrence of scalp defects, dysplastic ears, and absent or rudimentary nipples and breasts. Urinary tract malformations and/or disfunctions, and diabetes,

were reported in three cases.
We report the follow-up study in the affected one of two dyzygotic female twins, since the age of 2 years and during 36 years. The periodical clinical evaluation, with the recurrent, diagnostic, laboratory and instrumental tests, in order to follow the natural history, revealed a progressive number of additional features. Normal intelligence and behaviour, and the characteristic phenotype confirm the data reported in the very few cases of the literature. The patient was submitted to surgery correction of the congenital bald nodules over the scalp, reduction of the hypertelorism, correction of the prominent and dysplastic ears, and correction of the bilateral athelia and amastia. Unilateral renal hypoplasia, identified by ultrasounds at age 5 years is now associated with severe renal hypertension. Normal 46,XX constitutional karyotype and normal CGH array. By using a battery of polymorphic DNA markers we confirmed the DZ twinning.

P02.218
Cryptic chromosome 18 microdeletion encompassing SETB1 gene in a patient with expressive speech impairment and minor physical anomalies.

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Missense heterozygous mutations in SET binding protein 1 gene (SETBP1) have been recently demonstrated to cause Schinzel-Giedion syndrome (SGS, MIM #269150). SGS is characterised by severe mental retardation, distinctive facial features, and multiple congenital malformations abnormalities (skeletal anomalies, hearth defects, genitourinary and renal malformations) (Hoischen et al, 2010). More recently, some authors describes two patients with *de novo* chromosomal microdeletions in 18q12.3 and SETBP1 haploinsufficiency. The patients shows a milder phenotype distinct from SGS and characterised by moderate developmental delay and peculiar facial features including prominent forehead, sparse eyebrows, mild ptosis (Filges et al, 2010). Both patients exhibit expressive speech impairment. Here, we describe a patient with an apparently balanced *de novo* translocation t(2;18)(q24;q21) and mild mental retardation, peculiar facial features and expressive language impairment in presence of conserved receptive language skills. Oligo array-CGH analysis revealed the presence of a cryptic interstitial deletion of about 372 Kb. The microdeletion is located near the breakpoint region at 18q12.3 and causes the disruption of SETBP1 gene. The patient here described shows a significant phenotypic overlap with the previously reported patients with SETBP1 haploinsufficiency. All these patients differ significantly from the Schinzel-Giedion syndrome. The fundamental phenotypic feature of our patient and of the previously described two patients is the discrepancy between expressive and receptive language abilities, suggesting an essential role of SETB1 in expressive speech development. Functional studies will be necessary to unravel the exact function of this gene.

P02.219
A Large Turkish Setleis Syndrome Family

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Focal facial dermal dysplasias (FFDDs) are a group of rare ectodermal disorders characterized by bitemporal scar-like lesions that resemble forceps marks and additional facial findings that give an aged-leonine appearance. Three subtypes have been delineated based on clinical features. Lately, it has been found that homozygous nonsense mutations in TWIST2 cause type III FFDD, or Setleis syndrome.
We describe a Setleis syndrome family of Turkish origin in which there are three affected sibs born to a first degree cousin marriage. Detailed clinical features and pedigree will be included along with detailed imagery of characteristic facial findings. Mutational analysis are under way for TWIST2. These mutational data will be shared in the poster as well.

P02.220
Autosomal recessive form of Severe Combined Immune Deficiency Disease as the predominant pattern in Iranian patients

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Introduction : Severe Combined Immune Deficiency (SCID) is a rare syndrome which can show an X-linked or autosomal recessive inheritance pattern. World-wide investigations showed nearly 48% X-linked inheritance, caused by mutations of the IL-2 receptor. This form of SCID is characteristically $T^-/NK^-/B^+$. On the other hand many genes are involved in the autosomal recessive form. The aim of this study was to determine the inheritance pattern of SCID among affected families in Iran.
Methods: Genomic DNA of 20 unrelated male patients with T^-B^+ clinical symptoms of SCID was purified from peripheral blood. PCR and sequencing of the *IL2RG* gene was performed in patients to find out if they harbour any mutation.
Results: Because the patients were considered to have T^-B^+ SCID , we analyzed the *IL2RG* gene of the patients for mutations by direct genomic sequencing. The X-linked mode of inheritance was not detected in any patient. The *IL2RG* gene of all the patients was normal, suggesting an autosomal mode of inheritance of SCID.
Discussion: About 48% of SCID patients worldwide are reported to have IL2RG deficiency, but our preliminary result shows that the autosomal recessive pattern is predominant in Iranian SCID patients. The high frequency of consanguineous marriage makes it less likely that isolated cases of SCID are caused by *IL2RG* mutations. However, this conclusion is based on only a small number of patients. This is a pilot study and much more investigation is needed to clarify which gene is most frequently causative in Iranian patients.

P02.221
Molecular karyotyping identifies CNVs in individuals with short stature

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Shortness of stature is one of the most common concerns in genetic counselling. Overall, 3 % of the population present with a body height below -2 SD score (SDS). In about 80% of cases (idiopathic short stature) the underlying cause remains unknown.
To identify novel genetic causes of growth retardation we performed molecular karyotyping in 105 individuals with idiopathic short stature. We found a total of 3,787 aberrations with an average of 36 copy number changes per individual. To rule out copy number polymorphisms, a screening of all identified CNVs was carried out against an independent control cohort of 820 healthy individuals.
To narrow down our search for potential pathogenic CNVs, we analysed all remaining 1,499 aberrations based on their gene content. A total of 46 CNVs (27 duplications, 19 deletions) in 34 patients were retained for follow-up. Inheritance of 30 CNVs (2 *de novo*, 14 maternally and 14 paternally inherited) could be verified in 23 families. After re-evaluating the parental growth phenotypes, 10 CNVs (2 de novo, 6 maternally and 2 paternally inherited) in 9 families, including 5 duplications and 5 deletions, were scored as potentially pathogenic. These CNVs cover a range of 34 to 14,229 kb in size with only one aberration smaller than 100 kb.This is the first report of a systematic approach using molecular karyotyping to identify novel genetic causes of shortness of stature. We identified individual potentially pathogenic CNVs in 9% of affected individuals, supporting a “rare variant - frequent disease” hypothesis.

P02.222
SHOX gene polymorphic variants and their association with isolated short stature

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Mutations or deletions affecting production of the short stature homeobox-containing gene (SHOX) are responsible for a small portion of cases with isolated short stature (ISS). We were interested if *SHOX* gene polymorphic variants predispose for the ISS phenotype.
Our sample was made of 43 unrelated probands in charge of the Department of Medical Genetics (GTH and 1stFM, Charles University in Prague). Study inclusion criteria were the presence of short stature (-2,0 SD) in combination with at least one of following symptoms: disproportionate stature, cubitus valgus, short forearm, bowing of forearm, muscular hypertrophy, or dislocation of ulna (at elbow). A population sample of 96 individuals was used to compare the frequencies. DNA sequencing was done of *SHOX* gene exons 1 (including promotor), 2, 3, 4, 5 and coding parts of exons 6a and 6b.
Overall, eleven polymorphic variants (promoter, exon 1, exon 2, and exon 6b) were detected and one unique mutation in exon 6a. Four polymorphisms were already described in the SHOX database (SHOX @ http://www.hd-lovd.uni-hd.de/). Three promoter polymorphism and one sequence variant from the exon 1 are in a strong linkage. There was no statistically significant difference in rare allele frequencies between ISS group and population sample.
Our study indicates that *SHOX* gene polymorphic variants are not significantly associated with isolated short stature.
This work was supported by Grant No. IGAMZCR NS/ 10327-3/2009.

P02.223
Duplication of pseudoautosomal region 1 and *SHOX* gene could be linked with characteristic phenotype

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The reports on duplications of *SHOX* gene and pseudoautosomal region 1 (PAR 1) are scarce and thus the information on the possible phenotypic consequences is lacking. We report on five new patients from two families with large duplication of SHOX gene and downstream PAR 1 region. All of them were of normal or even short stature, with no signs of skeletal deformities or Mayer-Rokitansky-Kuster-Hauser type I syndrome. However all patients had a distinct phenotype, characterized with dysmorphic facial features (deep set eyes, high forehead, retrognathia), developmental delay/mental retardation, speech delay and stereotypic mouth movements which segregated with duplication of *SHOX* gene and PAR 1 in both families.
So far, data on only 4 patients with large duplications of both *SHOX* gene and PAR 1 downstream of the *SHOX* gene have been published. In three of them mental retardation and dysmorphic features similar to those observed in our patients were described.
Analysis of the size and position of duplications indicates that the ~50 kb large region of overlap between 626 kb and 678 kb, located in the PAR 1 could be responsible for the distinctive phenotype. It is very likely that duplications of *SHOX* gene and PAR 1, which still hasn't been completely sequenced, are under-ascertained. With increasing application of molecular cytogenetic methods like MLPA, more patients with large duplication of the *SHOX* gene and PAR 1 or isolated PAR 1 will be eventually discovered delineating phenotypic consequences in more details.

P02.224
Brachydactyly: a rare complication of sickle cell anaemia

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We present a 17 year old woman with acquired brachydactyly. The left hand showed a short metacarpal, proximal phalanx and mid phalanx of the fourth digit. There is also a deformation of the proximal phalanx. The right hand showed shortening of the third and fourth metacarpals. The pattern of the skeletal involvement did not fit with the known congenital brachydactyly groups. She was known with sickle cell anaemia and she endured dactylitis in childhood. In 40% of the cases with HbSS, the first clinical manifestation is dactylitis, also known as hand-foot syndrome. Dactylitis in sickle cell anaemia is the result of vaso-occlusion and infarction of the bone. In rare cases infarctions of the epiphyses result in an asymmetric brachydactyly, like in our case.

P02.225
11p15 familial duplication causing Silver-Russell syndrome in the daughter and Beckwith-Wiedemann syndrome in the mother.

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Silver-Russell syndrome (SRS) is a clinically and genetically heterogeneous disorder characterized by severe intrauterine and postnatal growth retardation. Beckwith-Wiedemann syndrome (BWS) is defined by overgrowth, hemihyperplasia and an increased risk of childhood tumors. It is known that SRS and BWS are both resulted by methylation defects in chromosomal region 11p15. Most of cases SRS and BWS are sporadic but some familial cases are also reported. We report here a familial case, where daughter has SRS and her mother BWS caused by duplication in 11p15 chromosomal region.
The proband was consulted by geneticist at the neonatal age due to dysproportionate intrauterine and postnatal growth retardation and dysmorphic features typical to SRS. We performed the chromosomal microarray analysis (CMA) of the child and found ~1.3-Mb size duplication in chromosomal region 11p15.5. CMA analysis of her parents revealed that the duplication is inherited from her mother, who has the same duplication at the region 11p15.5. Patients' mother has overgrowth since the birth and clinical features of BWS. The further investigations of grandparents are in work.
SRS and BWS have been linked with a variety of epigenetic and genetic defects affecting a cluster of imprinted genes at chromosome 11p15.5. More than 50% BWS patients have aberrant methylation patterns in 11p15. In SRS patients were found hypomethylation patterns in up to 63%; 11p15 duplication in maternal material was found in only 1-2%. There is previously only one report in the literature about 11p15 duplication resulted in SRS and BWS phenotype in single family.

P02.226
Molecular investigations of Polish patients with Silver-Russell syndrome

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Silver-Russell syndrome (SRS) is a clinically and genetically heterogeneous syndrome characterized by intrauterine growth restriction, body asymmetry, relative macrocephaly and a typical triangular face. SRS is caused by epigenetic dysregulation of the imprinted genes controlled by two Imprinting Centers Regions: ICR1 and ICR2 located on chromosome 11p15.5. Hypomethylation of ICR1 is found in ~40% of patients with SRS. In addition, hypomethylation of other imprinted loci can be detected in ~7% of ICR1 hypomethylation carriers. Moreover, 5-10% of SRS individuals have epigenetic alterations due to maternal uniparental disomy of chromosome 7 (mUPD7).
We analyzed 87 patients with clinical diagnosis of SRS. We examined the methylation profile of the ICR1 by MS-MLPA analysis and mUPD7 by STR analysis for chromosome 7. We confirmed hypomethylation of ICR1 in 31 individuals. Interestingly, 2 of these patients were monozygotic twins with both ICR1 and ICR2 hypomethylation. STR analysis carried out on patients without detected hypomethylation of ICR1 revealed mUPD7 in 8 patients. We demonstrated maternal hetero-, isodisomy and a mixture of hetero- and isodisomy. Applied molecular techniques enabled us to confirm clinical diagnosis of SRS in ~45% of patients. The cause of almost half of SRS cases is still unknown. It is hypothesized that other imprinted loci and molecular factors can be involved in SRS etiology. Our findings concerning ICR1 and ICR2 hypomethylation are consistent with the assumption.
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P02.227

Molecular karyotyping is indicated in growth-retarded patients with SRS features

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Silver-Russell syndrome (SRS) is a congenital disorder characterized by severe growth retardation, relative macrocephaly, a triangular face and asymmetry. The clinical spectrum is broad and the diagnosis is rather subjective. In nearly half of patients (epi)genetic alterations can be detected: >38 % show a hypomethylation of the imprinting control region 1 in 11p15; 10 % carry maternal uniparental disomy of chromosome 7 (upd(7)mat). Besides conventional cytogenetic findings recently submicroscopic chromosomal imbalances have been reported in single cases.To determine the relevance of submicroscopic imbalances for the aetiology of the SRS, we performed molecular karyotyping of 39 patients referred as SRS without 11p15 (epi) mutations and upd(7)mat using the Affymetrix SNP Array 6.0. In three patients probably pathogenic de-novo imbalances were identified: a 5.4 Mb microdeletion in 15q26 including *IGF1R*, a 2.5 Mb microdeletion of the DiGeorge region and a 9.1 Mb duplication in Xq26. In further 18 patients we detected a total of 26 different, so far unregistered copy number alterations (CNAs): up to now, 15 were determined to be probably apathogenic rare familial CNVs; for three CNAs a de-novo origin was discovered. To our current knowledge it is unclear whether they are causative for the patients' clinical features. In summary, in three of 39 (7,7%) patients pathogenic de-novo imbalances were detected. In further three patients the pathogenic significance of the CNAs remains unclear. In conclusion, submicroscopic chromosomal imbalances significantly contribute to the mutational spectrum of the SRS. Therefore molecular karyotyping is indicated in idiopathic patients with SRS features.

P02.228

A Simpson-Golabi-Behmel patient with severe neonatal liver involvement

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Patient prenatal period was marked by the discovery, on the 22 gestation weeks (GW) ultrasound of an hydramnios and fetal macrosomia (biparietal diameter and abdominal circumference >97 percentile). There was no gestational diabetes, and no fetal caryotype was performed. At 27 GW, a bilateral nephromegaly without pyelocaliectasis was found. A close ultrasound follow-up till the end of the pregnancy showed a stability of the ultrasound signs.

The patient was delivered by ceasarean section at 38 GW, and birth parameters were : weight : 4kg105, height 52cm and OFC 37cm. On examination, he had hypotonia, feeding difficulties and was icteric. He also presented facial dysmorphism with coarse features, hypertelorism, anteverted nares and macrostomia. Three abdominal supernumerary nipples and a abdominal diastasis recti were noted. Extremities were normal. No neonatal hypoglycemia was noted. Abdominal ultrasounds confirmed the bilateral nephromegaly, and then showed an hepatomegaly at 2 weeks of age. Blood tests showed a hepatic cholestasis and jaundice.

At the age of 6 weeks, because of worsening of cholestasis, persistent jaundice and difficulties displaying the biliary ducts on CT scan, a liver biopsy was performed. It showed no biliary atresia, but found a significant proliferation, portal fibrosis and cholestasis.

The study of the *GPC3* gene found a single base deletion in the exon 7, confirming the diagnosis of Simpson-Golabi-Behmel syndrome (SGBS).

Apart from the visceromegaly, neonatal liver involvement is very rarely associated to the SGBS. We will precisely describe this case, especially the hepatic data, and compare it to the literature.

P02.229

Expanding the Phenotype of Spondyloepiphyseal Dysplasia-Brachydactyly and Distinctive Speech syndrome in patients of Diverse Ethnic Backgrounds

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Spondyloepiphyseal Dysplasia-Brachydactyly and Distinctive Speech dysplasia (SED-BDS, MIM 611717) is characterized by postnatal short stature, brachydactyly, platyspondyly, facial dysmorphisms, delayed epiphyseal ossification and characteristic voice. Only a few cases have been described and the molecular etiology is uncertain. We present two unrelated individuals who share clinical and radiological features of SED-BDS and compare them with other conditions with similar phenotypes.

Patient 1 was born to a non-consanguineous Chinese couple. At 13 years of age he had severe short stature with height below the third centile. Dysmorphic features included upslanting palpebral fissures, mildly coarse face and brachydactyly. He has a high pitched hoarse voice were noted. Skeletal survey showed platyspondyly, metaphyseal irregularity and delayed bone age. Patient 2 was born a non-consanguineous Ghanian couple. He was diagnosed with congenital heart disease in infancy. At 7 years of age his height was below the third centile and had round face, brachydactyly and hoarse voice. Skeletal survey showed shortening of long bones with brachydactyly.

SED-BDS is a recently described skeletal dysplasia. Conditions that must be considered in the differential include acromicric and geleophysic dysplasias. Mutations in *ADAMTSL2* have been identified in some patients with geleophysic dysplasia and is thought to interact with TGF-β, which is important for cell proliferation, migration, differentiation, and survival. As these conditions have clinical and radiological overlap distinguishing between them can be a challenge. Molecular studies of *ADAMTSL2* in our patients are underway to further investigate the molecular defect underlying these disorders.

P02.230

Extense spanish pedigree affected of SMN1 gene deletion diagnosed by MLPA

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BACKGROUND: Spinal muscular atrophy (OMIM 253300) refers to a group of autosomal recessive neuromuscular disorders characterized by degeneration of the anterior horn cells of the spinal cord, leading to symmetrical muscle weakness and atrophy. SMA is the second most common lethal, autosomal recessive disease in Caucasians after cystic fibrosis. More than 95% of SMA patients show homozygous deletion of at least exon 7 of the telomeric *SMN1* gene. SMA carriers can be identified by the presence of only a single copy of *SMN1* exon 7.

MATERIAL AND METHODS: We report a three month-old infant who was hospitalized because she refused to eat. Clinical examination revealed an alert infant with weakness of all four limbs, hypotonia and absent deep tendon reflexes. Serum creatine kinase and liver enzyme studies were normal. Electrophysiological studies revealed reduced compound muscle action potential amplitudes (CMAPs). Distal latencies, F-waves latencies, and sensory nerve action potentials (SNAPs) were normal. Magnetic resonance imaging of the brain and spine was appropriate for age. A genetic test detected a complete deletion of the *SMN1* gene by MLPA. Members of the family were studied in order to detect carriers of the *SMN1* deletion.

RESULTS AND CONCLUSIONS: Genetic study of a *SMN1* deletion was performed in 21 members of both sides of the family. In the paternal family, a deletion was confirmed affecting *SMN1* and the *BIRC* gene, which is located close to *SMN1*. In the maternal family, the deletion was confined to the *SMN1* gene. We detected 16 heterozygous carriers for this deletion and proper genetic counseling was offered.

P02.231

A patient with short stature, microcephaly and mental retardation associated with 5q35.2 duplication reciprocal to the common Sotos syndrome deletion

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The higher-order architecture of the human genome has been shown to predispose to structural rearrangements that are frequent cause of human diseases. Recombination- and replication-based mechanisms have been described to generate genomic rearrangements. The mechanism responsible for the vast majority of common-sized recurrent rearrangements is nonallelic homologous recombination (NAHR) between region-specific low-copy repeats (LCRs). This mechanism assumes that recombination between the same pair of directly oriented LCRs could result either in deletion or reciprocal duplication. So far, only a few duplications reciprocal to the recurrent deletions associated with different syndromes (e.g. Williams-Beuren and Smith-Magenis syndrome) have been described.

Here we present a patient with 5q35.2 duplication of the Sotos syndrome critical region. The Sotos syndrome is characterized by childhood overgrowth, learning disability and distinctive facial appearance, and is caused by *NSD1* gene defects (point mutations, partial gene deletions and 5q35 microdeletions). Clinical evaluation of the patient at 13 years revealed short stature (-4SD), microcephaly (-3.5SD), dysmorphic face, bradydactyly, cone shape epiphyses, delayed bone age, and mental retardation. A 2-Mb duplication at 5q35.2 (5:174950741-176979615 (NCBI36)) was detected applying HumanCytoSNP-12 BeadChip (Illumina Inc.) analysis. So far, only two patients with duplications reciprocal to the Sotos common deletion have been reported, with phenotypes very similar to that observed in our patient. Thereby, our patient supports the hypothesis that *NSD1* plays a role in regulation of somatic growth in humans, and gene's dosage reduction or increase may lead to reversed phenotypes. More patients and further investigations are needed to reveal the exact mechanisms.

P02.232

Cutis laxa associated with Sotos syndrome: a clinical and molecular puzzle.

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Introduction. Cutis laxa, characterized by fragmentation of elastic fibres, has been associated with several syndromic phenotypes with heterogeneous mode of inheritance. Besides the skin symptoms, related systemic features are diverse, including skeletal, pulmonary, cardiovascular and metabolic symptoms.

Results & discussion. The first child of healthy non-consanguineous parents is presented, born at 38 weeks' gestation. During pregnancy, an isolated unilateral hydronephrosis was detected. At birth, dysmorphic features were noted, including macrocephaly with sparse frontal hair, a pointed chin and low-set, posteriorly rotated ears. In addition, redundant skin folds in the neck, hands and lower limbs were present. Skin biopsy confirmed the diagnosis of cutis laxa. Soon after birth, he developed episodes of hypoglycaemia. Radiographies revealed an advanced bone age (4.5 months for 5 weeks of age) and advanced bone maturation.

The phenotype of this patient showed resemblance to Costello syndrome. However, these patients do not have advanced bone age and maturation. Micro-array analysis revealed a 5q35.2q35.3 deletion, compatible with Sotos syndrome. Cutis laxa is however not a classic feature. Three cases of alleged Sotos syndrome with cutis laxa have been previously reported; however, contrary to our patient, neither advanced bone age nor hypoglycaemia were present.

Conclusion. The phenotype of our patient does not fully comply with the Sotos with cutis laxa syndrome. This might suggest that this patient harbours a second mutation in the deleted 5q35 region which may explain the presence of cutis laxa.

P02.233

Intrafamilial variability in a large kindred with SVAS and an *ELN* mutation

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Supra Valvular Aortic Stenosis (SVAS; MIM ID #185500) has an incidence of 1/20 000 live births and is associated with mutations in elastin gene (*ELN*). SVAS can occur sporadically, as a dominant trait or as a part of Williams-Beuren syndrome (WBS). WBS is characterized by developmental delay, particular psychological phenotype and dysmorphic features, and is caused by a microdeletion ~1,5Mb at 7q11.23 which encompasses *ELN* and at least other 24 genes.

We report on a large family with 17 affected members spanning 4 generations with variable phenotypic presentation with isolated or a combination of several major arteries stenosis, namely: SVAS, common pulmonary stenosis or peripheric pulmonary stenosis. No developmental delay, particular psychological phenotype or dysmorphic features of WBS were present in any of the affected individuals.

ELN exons and intron-exon boundaries were amplified by PCR and mutation screening was performed by High Resolution Melting (HRM). Exons showing an abnormal amplification profile or with frequent SNPs were sequenced. After testing 18 coding exons, a heterozygous transversion G>T was found at position 34 (c.34C>T) changing the Glycine at position 12 to a stop codon (p.Gly12X). This nonsense mutation is presumably responsible for haploinsufficiency as often observed in *ELN* mutations in SVAS.

Remaining affected members are currently being investigated for the mutation what will expectably confirm segregation within this family.

This report underlines the clinical intrafamilial variability of *ELN* mutations and highlights the importance of cascade screening enabling a proper genetic counseling.

P02.234

A six year old boy with Temple syndrome due to segmental maternal uniparental disomy 14 and clinical features of Prader Willi syndrome

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Temple syndrome is associated with a recognizable phenotype consisting of pre- and postnatal growth retardation, neonatal hypotonia, feeding problems and precocious puberty. The molecular cause of Temple syndrome comprises a deficiency of imprinted gene expression in the paternal chromosome region 14q32 due to maternal uniparental disomy 14 (upd(14)mat), an epimutation or deletion on the paternal allele. Chromosome 14 contains an imprinted gene cluster, which is regulated by a differentially methylated region (IG-DMR) between the genes *DLK1* and *GTL2*.

We report on a six year old boy with severe neonatal hypotonia and feeding difficulties, followed by hyperphagia with onset during his third year of life with absence of satiety and leading to obesity. Additional features were short stature, small hands and feet, delayed psychomotor development, sleep apnea and fever attacks. His phenotype was strongly suggestive for Prader Willi syndrome, but methylation analysis of the 15q11-q13 region revealed no abnormalities. Considering Temple syndrome as an important differential diagnosis we consequently performed methylation analysis of the 14q32 region, which revealed lack of the paternal band. Further analysis with microsatellite markers and SNPs detected a segmental upd(14)mat of the 14q32 region in our patient.

To our knowledge, there are no reported patients with a segmental upd(14)mat of the 14q32 region so far. We provide a review of the literature on patients with Temple syndrome and discuss on the phenotype genotype correlation.

P02.235
Partial tetrasomy 14 associated with multiple malformations

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We report a 6-year-old girl with multiple malformations including bilateral cleft lip and palate, coloboma and craniosynostosis. The girl also suffers from severe mental retardation, seizures and gastrointestinal dysfunction. In addition she has a mitochondrial defect with reduced function of complex I and III of the respiratory chain. An abnormal karyotype was identified showing an extra marker chromosome. The marker chromosome was identified in all cells in both peripheral blood lymphocytes and cultured fibroblasts whereas parental chromosomes were normal. Array-based comparative genomic hybridization (CGH) revealed tetrasomy for proximal 14q corresponding to a 14,6 Mb region on 14q11.2q13.2 spanning more than 60 known genes. To determine the origin of the marker chromosome we analyzed polymorphic markers using quantitative fluorescent PCR (QF-PCR). The results from the QF-PCR indicate formation of the marker chromosome in maternal meiosis. Partial tetrasomy 14q has previously been reported in four cases, however, this is the first described non-mosaic case not resulting in early lethality.

P02.236
The incidence and morbidity of congenital thoraco - abdominal wall defects

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Background: Congenital abdominal and thoracic wall defects remain a source of significant morbidity and mortality, despite the advances in neonatal and pediatric surgical care. The abdominal wall is much more often involved in congenital birth defects than the thoracic wall, which are more severe and have dismal outcomes more often. Material and methods: This is a 4 years period analysis of the entire cases of congenital thoracic and abdominal wall defects admitted to the pediatric surgery department of our hospital. Results: There were six cases of gastroschisis, two omphaloceles, one sternal cleft and one case of ectopia cordis. Surgical repair was performed in emergency conditions for all gastroschisis and omphalocels with good outcomes. Postoperative complications were: intestinal adhesions after one case of gastroschisis repair and rezidual eventration after omphalocele closure, considered with no significant influence over the final outcomes. For ectopia cordis surgical covering was performed but, unfortunately the patient died 24 hours after surgery. No surgical treatment was necessary for the sternal cleft. Conclusions: Although the prenatal diagnosis is accessible, there are many cases diagnosed after birth. This study helps to bring together the most recent epidemiological data regarding outcomes and interventions for thoracic-abdominal wall defects and to compare the epidemiology of such kind of malformations in different regions of the country.

P02.237
Three Tumor Necrosis Factor promoter polymorphisms in Romanian patients with Sjogren's syndrome - Results from a pilot-study

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Sjögren's Syndrome (SS) is an autoimmune disorder which affects exocrine glands. Tumor Necrosis Factor alpha (TNF-α) contributes to the physiopathology of autoimmune diseases including SS. The -857C/T, -308G/A and -238G/A polymorphisms of TNF-α gene demonstrated their influence on the susceptibility and clinical phenotype of autoimmune diseases.
We aimed to investigate the influence of these three TNF-α polymorphisms on the susceptibility and clinical phenotype of primary Sjögren's syndrome in Romanian patients.
We assessed 141 Romanian subjects (37 primary SS patients and 104 healthy unrelated matched controls) for all three polymorphisms. TNF-α -857C/T (rs1799724), -308 G/A (rs1800629) and -238G/A (rs 361525) polymorphisms were genotyped by Real Time PCR (Taqman SNP Genotyping Assays C_2215707_10, C_7514879_10 and C_11918223_10 respectively, Applied Biosystems, USA). Association tests for each SNP were performed using SPSS 12.0 (T student test) and p values ≤0.05 were considered significant.
The Hardy-Weinberg equilibrium assessed using Chi-square test was respected in all studied groups for all polymorphisms.
The frequencies of minor alleles -857T, -308A and -238A, were similar in primary SS patients and controls (0.21/0,16/0.09 and 0.18/0,15/0.04 respectively). Primary SS patients carriers of A allele (-238G/A) seems to have less digestive (t= 2,95) and systemic (t=1,4) manifestations than G carriers. The primary SS patients carriers of T allele (-857C/T) seems to have more xerophthalmia (t=2,8) and xerostomia (t=3,04) than C allele carriers.
Conclusion: TNF-α polymorphisms (-857C/T and -238G/A) seems to influence clinical manifestations. The three polymorphisms of TNF-α does not influence susceptibility to SS. These results should be confirmed on larger patients cohort.

P02.238
Unusual family advice causes Turner-like syndrome

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Objective: To recognize long term use of high potency steroids from an early age as a potential cause of iatrogenic Cushing Syndrome (CS) and Turner-like symptoms.
Case Presentation: A 16 year old teenage girl was referred from her gynecologist with diagnosis of Turner syndrome (TS). The most consistent features of TS are short stature and premature ovarian failure. She had initially presented for follow up of amenorrhea about 8 weeks prior to referral and was noted to have short stature (<3.p) at a hospital. Upon presentation to our clinic, she was noted to have a round plethoric face, supraclavicular/dorsocervical fat pads, centripetal obesity and short stature. Lab tests showed ACTH 15pg/mL (6-46pg/mL) and 8 a.m cortisol of 0.3mcg/dL (6-30mcg/dL). TSH, LH, FSH, and prolactin were normal. Chromosomal analysis revealed a 46,XX karyotype. Iatrogenic and factitious Cushing Syndrome was investigated. Further discussion with the patient and her family revealed oral use of 0.75 mg/day dexamethasone for 12 years. The patient is a 16 year-old teenage girl who had begun to take high dose deksamethasone by the advice of her family at the age of four. She continued to use the steroid so far.
Conclusions: Oral intake of high dose steroid at the beginning of the childhood can cause iatrogenic CS and Turner-like symptoms. An inspection is not enough searching for the right diagnosis of genetic syndromes. We recommend a careful patient history and a detailed physical examination in terms of Turner syndrome and other genetic diseases.

P02.239
Unbalanced chromosomal translocation 3;5 in child with dysmorphic features and developmental delay. Case report.

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We report a clinical case of a unbalanced translocation between 3p and 5q chromosome terminal regions which results in 3rd chromosome short arm subtelomeric region deletion and 5th chromosome long arm subtelomeric region trisomy.
Consulted for the first time at the age 4y6m due to speech delay and changed behavior. Anamnesis: boy was born from 5th pregnancy, 3rd delivery to 38 years old mother from nonconsanguinous marriage. During first trimester mother had flu. Delivery on 38/39th week of gestation, birth weight 2360g, length 49cm. He had poor weight gain during 1st year of life, he started to walk at the age of 1y4m. He had one episode of seizures at the age 4y 5m. Speech- he use less than 10 words. Hearing test has not been performed.
Investigation: height 96cm (<<3rd percentile), weight 14kg (3rd pc), OFC 46.6cm (<<3rd pc), 6.5cm, 3rd finger 4.5cm. Dysmorphic features: deformed skull, almond shaped eyes, small mouth, irregular tooth placement, smooth philtrum, over folded helix, fetal pads, changed

dermatoglyphics in left hand, unilateral cryptorchism. Behavior: comes to sit in lap and wants to kiss during consultation, puts hair and dog fur into mouth, touches himself, no toilet training.
There was performed a karyotyping and FISH for subtelomeric region. Result: 46,xy.ish der(3)t(3;5)(pter-;qter+). Karyotyping was performed to both parents and father is a carrier of balanced translocation with result- 46,xy,t(3;5)(p26;q32~33).

P02.240
Clinical expression of an inherited unbalanced translocation in Chromosome 6

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Congenital malformation in newborns is the first visible expression of major chromosomal alterations followed by growth and mental retardation and systemic complications. The unbalanced rearrangements are not common; however, have significant clinical expression. The parental balanced translocation produces unbalanced chromosome, which is eventually transmitted to next generation through fertilization of gametes carrying the derivative chromosome. The carriers of balanced rearrangements mostly do not have recognizable phenotypic expression. We report a family comprising of healthy and non-consanguineous young parents and their primi newborn severely affected with multiple congenital anomalies and systemic disorders. Conventional G-banding analysis of somatic chromosomes identified a balanced translocation, t(6;10)(p23;q24) in mother and an unbalanced rearrangement, der(6),t(6:10)(p23;q24)mat in the child. The child has inherited a derivative chromosome 6 with partial deletion of 6 (p23-pter) and partial trisomy 10(q24-qter), which has obviously resulted in fusion of genes of two different chromosomes. The prominent phenotypic features of del(6p), including high forehead, flat nasal bridge, agenesis of left year, atrio septal defect (ASD), craniosynostosis and growth retardation were overlapping with specific Axenfeld-Reiger -, Larsen - and Ritscher-Sinzel/3-C syndromes; however, lacking in ocular anomalies, skeletal laxity or cerebellar malformation. Therefore, this report rules out the isolated effect of del(6p23) or trisomy 10(q24) on distinct previously reported syndromes and proposes the combined effect of unbalanced chromosomal alteration.

P02.241
Paternal uniparental disomy (UPD) 14 is an important cause of infantile hypotonia in a South African cohort

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Paternal and maternal uniparental disomy (UPD) for chromosome 14 are associated with two distinct phenotypes, accounted for by two oppositely imprinted genes on chromosome14, *DLK1* (paternally expressed) and *MEG3* (maternally expressed). Maternal UPD 14 is characterised by intrauterine growth retardation, hypotonia at birth, short stature, small hands and feet, scoliosis, mild developmental delay, early childhood obesity and precocious puberty, a phenotype overlapping with Prader-Willi syndrome (PWS). Paternal UPD (14) is reportedly less common, more severe and is characterised by severe neonatal respiratory problems, hypotonia, abdominal muscular defects, skeletal anomalies, and characteristic facies.
The study aimed to determine the frequency of UPD14 in 238 patients who were referred for PWS (86 patients) or SMA (152 patients) testing and who tested negative for the requested disease. Patients were sub classified into an infantile hypotonia group and a childhood PWS phenotype group. Genomic DNA was modified with a bisulfite modification kit. Multiplex methylation PCR of the modified DNA was used to determine whether UPD14 was present. Thirteen (5.5%) patients tested positive for paternal UPD14, while no patients tested positive for maternal UPD14. All paternal UPD 14 patients were from the hypotonic infants group. Further clinical and molecular analysis is in progress.
Paternal UPD14 which is reported to be rare was identified in a significant number of infants (<1 yr) referred for PWS and/or SMA testing, suggesting that neonatal and infantile hypotonia is a significant feature. UPD 14 analysis should be included in the routine genetic workup of patients with infantile hypotonia.

P02.242***
Wang syndrome- Four new cases each with a different underlying molecular mechanism

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Background
Wang syndrome is the eponym for paternal uniparental disomy for chromosome 14 (UPD14pat). Affected children present with polyhydramnios, narrow thorax with characteristic coat-hanger ribs, hypotonia, abdominal wall defects, high birth weight but poor postnatal growth, developmental delay, and typical facial dysmorphism including prominent, hisute forehead, elongated philtrum, micrognathia, short neck and mild joint contractures. We report four new cases of Wang syndrome with different underlying molecular mechanisms.
Report
Polyhydramnios and relatively increased abdominal circumference were noted during pregnancy in all cases. All patients showed characteristic chest x-ray and clinical features of Wang syndrome in the neonatal period. Methylation-specific PCR of the 14q32.2 differentially methylated region (DMR) showed only a methylated (paternal) product in all cases.
Case 1: Male child with an epimutation at maternally inherited 14q32.2 DMR.
Case 2: Female infant with segmental UPDpat for the 14q32.2 region.
Case 3: Male infant with a de novo microdeletion of the 14q32.2 DMR on his maternally inherited chromosome 14.
Case 4: Female infant with isodisomy for paternal chromosome 14.
Conclusion
These cases demonstrate that Wang syndrome is a clinically recognisable phenotype with typical radiological findings. Further studies are required to delineate the long-term prognosis and correlate this to the underlying molecular mechanism.

P02.243
Van Maldergem syndrome - a report of the first male patient and expansion of the clinical spectrum.

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Two unrelated female patients with a combination of mental retardation, hypotonia, distinctive combination of minor facial anomalies with telecanthus, epicanthus, broad flat nose, large mouth with an everted lower lip, small dysplastic ears, finger camptodactyly, and joint hyperlaxity were described by van Maldergem et al (1992) and Zampino et al (1994).
We report on the first male patient with the proposed diagnosis of van Maldergem syndrome. His parents are first cousins.
At the age of 4 years the boy presented severe developmental delay, talipes equinovarus, genital abnormalities, finger camptodactyly with interdigital webbing, joint laxity and a dysmorphic facies. The medical history was significant for pharyngeal instability requiring the placement of a tracheostomy tube and an inquinal hernia. He showed bilateral epicanthus, telecanthus, short palpebral fissures, broad flat nasal bridge, dental malocclusion, bilateral microtia. Due to severe feeding difficulties permanent tube feeding is required. The combination of the specific facial features with camptodactyly, interdigital webbing, joint laxity and developmental delay lead to the working diagnosis of van Maldergem syndrome. Metabolic tests and chromosomal analysis were normal. Array-CGH (Agilent 2x400K) revealed two parental CNVs, which are not listed as polymorphisms but might contribute to the patient's phenotype. Taking together, the report of the third patient with van Maldergem syndrome adds new features to the clinical spectrum such as male genital malformations, hernia, pharyngeal instability and severe feeding difficulty.

P02.244
A case of adult-onset vanishing white matter disease with *EIF2B3* gene mutations

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Leukoencephalopathy with vanishing white matter (VWM) is a rare neurological disease associated with gene defects in *EIF2B1-5*. It is an autosomal recessive disorder with spasticity, cerebellar ataxia, and a relatively mild mental impairment. In most reported cases, *EIF2B5* mutations were identified. This is the first report of a patient with adult-onset VWM associated with mutation in *EIF2B3*.

A 31-year-old woman with progressive ataxic gait was referred. At age 20, an MRI study had shown diffuse leukoencephalopathy in the absence of any clinical neurologic signs. Five years later, headache and sensory disturbance appeared. At age 28, amenorrhea and ataxic gait appeared. All laboratory investigations, including evaluation of blood lactate and pyruvate,α-galactosidase and arylsulfatase A, were normal. Genetic study confirmed the diagnosis, revealing that the patient was homozygous for the novel T80A mutation in *EIF2B3*. By age 31, she has become bed-ridden.

P02.245
Waardenburg syndrome: Intrafamilial clinical variability in a 4-generation family.

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Waardenburg syndrome (WS) is a rare syndrome with estimated prevalence of 2-3/100,000 in the general population within the Netherlands. Main features include facial dysmorphisms and congenital deafness. WS is classified into several forms based on whether dystopia canthorum is present (WS1) or absent (WS2), and whether upper limb defects (WS3) or Hirschsprung disease (WS4) are present. WS1 exhibits autosomal dominant inheritance with complete penetrance and variable expressivity. Mutations in PAX3 gene are responsible for WS1 phenotype. The index case is a 4-year-old female, first child of a young non-consanguineous couple, presented with displacement of the medial canthi, heterochromia iridis, white forelock and bilateral congenital sensorineural hearing loss. Her family history, however, showed other 06 affected relatives with different features of WS1.The purpose of this report is to describe the clinical variability among the affected members of this family. Molecular analysis is important to confirm the diagnosis and may help determining genotype-phenotype correlations.

P02.246
Knockouts of the murine ortholog of the atypical Williams-Beuren tyrosine-protein kinase gene Baz1b present cardiac and circadian rhythm impairments

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The Williams-Beuren syndrome (WBS) is a multisystem disorder caused by the hemizygosity of 25 genes, five of which encode transcription factors or chromatin remodeler. Modest perturbations in the dosage of the latter might substantially alter the expression of multiple target genes. Hence these genes are prime candidates to play a role in the phenotype of the patients.

To address the physiological and molecular role of one of these genes, the Bromodomain Adjacent to Zinc finger 1b gene (*BAZ1B*), *in vivo*, we ablated by transposable element insertion the expression of its ortholog in mice. Almost all *Baz1b*$^{-/-}$ embryos (97%) die a few hours after birth. The rare survivors do not present any of the craniofacial abnormalities previously identified in knockouts of the murine orthologs of other genes of the WBS interval. They are, however, smaller in size than their control littermates and present a smaller heart with larger ventricular walls. Noticeably, this abnormal heart appears to be associated with an increased cardiac function as measured by the percentage of blood ejected with each contraction. This translates in a significant increase in nighttime activity and sleep perturbation. We are currently characterizing histologically the heart of *Baz1b*$^{-/-}$ embryos.

Potential candidates genes for these multiple phenotypes have been identified by chromatin immunoprecipitation with antibodies raised against the BAZ1B unusual tyrosine-kinase.

P02.247
Pyrroline-5-carboxylate reductase 1 deficiency leads to a complex clinical phenotype; Report of 6 patients

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Wrinkly skin syndrome is a heterogeneous condition characterized by cutis laxa, wrinkly skin over hands and feet, progeroid appearance, visible veins over thorax, congenital hip dislocation with or without mental retardation. Loss of function mutations in the ATP6V0A2 gene and mutation in the gene encoding pyrroline-5-carboxylate synthetase (ALDH18A1; 138250), has been reported in some patients diagnosed with wrinkly skin syndorme.

Recently, mutations in *PYCR1* were described in patients with similar features (Guernsey et al. 2009; Reversade et al. 2009). The pathophysiological basis appears to be an impaired mitochondrial function leading to developmental defects through increased apoptosis. Here, we describe six patients with clinical manifestations of a wrinkly skin disorder. The patients all have distinct facial features comprising of thin triangular face, loss of adipose tissue, and thin pointed nose. Additional features are IUGR, short stature, wrinkling over dorsum of hand and feet, visible veins over chest, hyperextensible joints (mostly of fingers and toes), congenital hip dislocation, loss of muscle bulk. The age ranges from 4 months to 55 years. Three of the patients from a large consanguineous family did not have mental retardation while the remaining three patients from 3 separate families had mental and or developmental delay. Mutation analysis revealed the presence of disease-causing variants in *PYCR1*. Light- and electron microscopy investigations showed alterations of collagen and elastic fibres compatible with the involvement of collagen and elastin in the pathophysiological mechanism.

P02.248
Giant congenital melanocytic nevi mistaken for neurofibromatosis type 1

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An 80-year old developmentally normal male was referred to our department with a clinical diagnosis of neurofibromatosis type 1. The patient was born with numerous diffuse lenticular erythematous and brownish firm papules covering his entire body. The papules differed in size from several millimeters to one giant plaque covering almost his entire back. At the age of one year, part of this lesion was removed from his neck in order to allow better movement of his head. Over the years two more papules were removed. Removed papules did not grow back. His medical history showed basal cell carcinoma which was diagnosed on two occasions. He had an otherwise unremarkable medical and family history. His two sons and grandson were reported not to be affected by this disorder. Upon examination, no freckling, café-au-lait spots or Lisch noduli were noted and testing of the *NF1*-gene did not reveal a mutation. Array CGH was normal. Pathology results showed that the skin lesions were nevi naevocellulares, fitting the diagnosis of giant congenital melanocytic nevi (GCMN). GCMN are disfiguring lesions, consisting of cutaneous melanocytes. These GCMN, presenting at birth and covering minimally 2% of the total body surface area, are uncommon and have an estimated prevalence of one in 20.000 in live born children. GCMN usually occur sporadically, but familial cases have been described. The genetic background however remains unknown. To our knowledge this is the first time a case with such an extensive and unusual presentation of GCMN is described.

P02.249
Identification of a WWOX germinal mutation in a Spanish family without diagnosed cancer: insights into its supposed tumour suppressor function

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The WW domain-containing oxidoreductase (*WWOX*) gene is located at 16q23.3-q24.1, within the fragile site FRA16D. It has nine exons and an open reading frame of 1,245 bp, encoding a 414 amino acids protein.

In the last few years, considerable amount of data has shown that *WWOX* participates in a number of cellular processes including growth, differentiation and apoptosis. Moreover, inactivation of *WWOX* has been observed in a variety of human malignancies, and targeted deletion of one allele of *Wwox* in mice causes increased spontaneous and chemically induced tumour incidence, including lymphomas, lung papillary carcinomas, liver tumours, and gastric squamous cell carcinomas. Altogether, these data indicate that *WWOX* is a tumour suppressor and that inactivation of one allele is sufficient for tumourigenesis.

Here we report the identification of a *WWOX* germline mutation in a 52-years-old female with psoriasis and an extradural mass (neurogenic tumour versus hemangiopericytoma or meningioma were considered as differential diagnosis), who was referred to our laboratory for molecular testing of Neurofibromatosis 1. The mutation consists in a 48.6 kb deletion and a 14 bp insertion, resulting in exclusion of exons 6-8 from the mature transcript. The same mutation was detected in the proband's father and two siblings, without diagnosed cancer.

Our findings show that inherited mutations in *WWOX* gene do not lead to a high predisposition to develop malignancies, as could be expected from mouse models and other reported data. Future identification of more families with germinal mutations in *WWOX* will allow clinicians to define its associated phenotype.

P02.250
Maternally inherited duplication Xq11.1-Xq13.1 in a boy with craniosynostosis, mild mental retardation and facial dysmorphism

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Partial duplications involving the long arm of chromosome X have been reported as causing mental retardation and multiple malformations. Although data regarding these specific chromosomal anomalies in males are limited and duplication extensions are variable, a clear pattern of characteristic features, including short stature, developmental delay, cryptorchidism, restricted joint mobility and cranio-facial dysmorphisms has been identified.

We report the case of a 14 year old male patient with craniosynostosis, mild mental retardation, facial dysmorphisms and non specific polyarthritis. Chromosomal analysis on peripheral blood lymphocytes showed a normal male karyotype. Array-CGH, MLPA and quantitative PCR analysis revealed a duplication of approximately 5.9MB on the long arm of the X chromosome, subsequently identified in the proband's mother and grandmother.

As far as we know this is a unique rearrangement as there are no previous reports about a Xq11.1-Xq13.1 duplication. Among the 35 duplicated genes, *EDA* appeared to be the best candidate to explain at least some of the phenotypic peculiarities in our patient being the only gene known to have been associated to a gain of function phenotype in mice. It encodes ectodysplasin, a TNF- family protein required for normal morphogenesis and differentiation of several ectodermal organs. We therefore analysed the levels of *EDA* transcript and some of its downstream interactors by quantitative PCR.

Our data showed marked hyperexpression of *EDA*, supporting its role in determining, at least partially, the phenotype of our patient. Dosage sensitive genes as cause of the phenotype of our patient will then be discussed.

P02.251
A case of Zimmemann-Laband syndrome with supernumerary phalanx

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Zimmermann-Laband syndrome (MIM 135500) is the association of gingival fibromatosis, aplasic or hypoplasic nails, skeletal anomalies, hypertrichosis and facial dysmorphism. Occasionally, patient can display mental retardation, hepatosplenomegaly, mild hearing loss and supernumerary teeth. Molecular basis is still unknown but pattern of inheritance is more likely autosomal dominant.

We report on a 7 year-old girl with the hallmark of Zimmermann-Laband syndrome and particular distal phalanges. A large spectrum of phalangeal abnormalities could be seen such as classical hypoplastic but also epiphyseal hypertrophy, bi-partita or supernumerary phalanx. We discuss the spectrum of skeletal anomalies in this syndrome. In fact, if gingival and oral findings are frequently and well described, skeletal aspects of this disease are less known.

Cytogenetic exploration and CGH-array have been practiced on this patient's blood sample and results will be discussed.

P02.252
Clinical spectrum of heritable disorders connective tissue in Russian cohort patients

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Background: Heritable disorders of connective tissue (HDCT) often predispose patients to aortic aneurysm and acute aortic dissection. Forms of HDCT caused by mutations in the *TGFBR1* or *TGFBR2* genes have a severe prognosis due to the high risk of aortic rupture.

Patients and Methods: We performed clinical and genealogical examination of patients undergoing surgical repair of aortic root dilation/aneurysm and their relatives. Personal and family history were collected, and clinical examination (standard and 24-hour Holter ECG, EchoCG, Dopplerography, chest radiography) and genetic analysis were performed.

Results: We examined 66 index patients and relatives (mean age 33 y.o., range 4 to 64 y.o.) with HDCT symptoms (57% male). A positive family history was detected in 21%. A significant number of patients (77%) required operation because of large vessel aneurysm and/or valvular insufficiency. The phenotypic spectrum within the HDCT group was as follows: Loeys-Dietz Syndrome 5%, Marfan Syndrome 16%, Ehlers-Danlos Syndrome type IV 14%, Ehlers-Danlos Syndrome type VI 4%, TAAD 26%, functional valvular insufficiency 11%, aneurysms of large vessels 7%, MASS phenotype 3%, non-differentiated HDCT 14%. The overwhelming majority of patients had had cardiac arrhythmias. Mutation screening in the *TGFBR1* and *TGFBR2* genes is in progress now.

Conclusion: Correct molecular confirmation in families with HDCT is required to identify probands at high risk for aortic dissection. Even in the absence of identified mutations causing HDCT, we recommend standardized examinations of first-degree relatives in affected families. Prophylactic aortic root replacement should be considered for patients at risk.

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P02.253
A case with Noonan syndrome and renal hypoplasia

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Introduction: Noonan syndrome comprises an unusual facies and multiple malformations, including congenital heart disease. Aim: To present the case of a boy with Noonan syndrome and right renal hypoplasia. Material and Methods: 19 months old B.M., of male gender, was referred to our department for a urinary tract infection. We

based our diagnosis on clinical features and imaging studies. Results: During the physical we noted the following features: micrognathia, low-set posteriorly rotated ears, hypertelorism, down-slanting palpebral fissures, epicanthal folds, proptosis, strabismus, dental malocclusion, short neck with low posterior hairline, shield chest, pectus excavatus, brachydactyly, blunt fingertips, retractile left testis. Furthermore, the child had a short stature. The neurologic consult found a mild motor delay. The cardiac ultrasound showed aortic regurgitation and obstructive hypertrophic cardiomyopathy. We performed an abdominal ultrasound which revealed right renal hypoplasia. The audiologic and ophthalmologic examinations were unremarkable at this time, but the child will require ongoing follow-up. Discussion: Renal anomalies are present in 10% of patients and are not usually clinically significant. In this case, a condition associated with this anomaly led to the proper diagnosis. Conclusion: Although described for the first time almost 50 years ago, Noonan syndrome can still reveal itself in unusual circumstances.

J02.01
Deafness- associated DFNB599 gene (Pejvakin) mutations in Sistsn va Bluchestan province.

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Background and aim: Hearing loss is a common disorder affecting millions of individuals worldwide with opproximately 1 in 1000 newborns. A novel gene, DFNB59 encods Pejvakin has been recently shown to cause neural deafness. The aim of this study was to determine the frequency of DFNB59 gene mutations in 93 deaf pupils in Sistsn va Bluchestan province.
Methods: We investigated the frequency of DFNB59 gene mutations in the coding regions (exons 2-7) of the gene.
DNA was extracted following the standard phenol chloroform procedure, the frequency of DFNB59 gene mutations was investigated using PCR-SSCP /HA strategy.
Results: No pathogenic variant was detected in samples studied. However, one polymorphism including 793C>G was determined in 3 of 93 (3.2%) subject examined.
Conclusion: The results of this study showed no associated between DFNB59 gene mutations and hearing loss in Sistan va Baluchestan province.

J02.02
New mutation in Notch3 gene of CADASIL in Iranian patients

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Cerebral autosomal dominant arteriopathy with sub cortical infarcts and leukoencephalopathy (CADASIL (is an inherited cerebrovascular disease due to mutations of the Notch3 gene at the chromosome locus 19p13. The clinical spectrum includes recurrent ischemic episodes, cognitive
Deficits, migraine and psychiatric disorders. MRI reveals extensive cerebral white matter lesions
And sub cortical infarcts. Notch 3 gene, encodes a transmembrane receptor and contains 33 exons. The mutation responsible for CADASIL are located within the extracellular domain of Notch 3, with strong clustering within exon 3 and 4 in more than 70% of patients.
In this study DNA was extracted from a patient suspected to CADASIL with clinical manifestations. Exon 3 and 4 of Notch 3 gene was amplified by PCR. After sequencing we found a c.347A>T mutation in exon 4 0f Notch 3 gene .This mutation alters Aspartic acid to Valine..

J02.03
The first case of diagnostics mucopolysaccharidosis type III in Novosibirsk region.

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We report a case of diagnosis of Sanfilippo syndrome, the first in the territory of the Novosibirsk region. The diagnosis was made at a medicogenetic consultation at a children's psychoneurological dispensary. The proband, a girl of five years, was observed by the psychiatrist with a diagnosis of serious mental retardation (F72).
Psychoneurological features were prominent. In development of the child there was a retrogression of mental functions with a crushing defeat of intelligence, speech and communication functions, disturbances of behaviour with excitation and aggression. A lack of skills of self-care becomes perceptible. Craniofacial dysmorphism and signs of an osteal dysostosis were expressed moderately. The neurologic status: a lower spastic paraparesis, Babinsky positive signs, disturbance of gait and coordination, a divergent squint.
Functional examination showed CT signs of hydrocephaly, signs of an intracranial hypertension; a thickening of the cusps of the mitral valve, a sinus arrhythmia and episodes of transient sinuatrial blockade.
The clinical diagnosis has been confirmed in the laboratory of hereditary diseases of metabolism of the RAMS Research Center for Medical Genetics (Moscow). In the *SGSH* gene the mutation p.R74C was identified. Distinctive features of the present case are absence of the cramps characteristic of the classical variant of MPS III, neurosensory deafness and diarrhoea. It is possible to assume that this is related to the early diagnosis of disease.

J02.04
Pierre Robin Syndrome - Evolution and Prognostic at Distance

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Introduction: Pierre Robin syndrome is a rare condition in current medical practice, with an incidence of 1/8500-1/30000.
Clinical diagnosis is easily accomplished in the presence of typical associated malformations.
Objectives: We present the case of a newborn with Pierre Robin syndrome associated with omphalocele in major form.
Material and method: A premature newborn, gestational age 35 weeks, female, from a pathological pregnancy, presented at birth with a major omphalocele, requiring rapid surgical intervention. The particular phenotype with severe micrognathia, cleft palate, glossoptosis associated with stuck sternum and intraventricular septal defect easily suggested the clinical diagnosis.
Results: Low birth weight, early surgical intervention, cardiac malformation, associated respiratory distress and abdominal wall defect made a difficult clinical evolution. There were no karyotypic abnormalities. Progress was difficult due to feeding difficulties and recurrent respiratory infections that led to weight loss and prolonged hospitalizations.
Conclusions: 1. Clinical diagnosis of Pierre Robin syndrome is easily effected. 2. Even though severe associated malformations were present there were no karyotypic abnormalities.
3. The long-term prognosis is good through treatment and management by a multidisciplinary team.

J02.05
Prevalence of the A1555G, A3243G and A7445G mitochondrial mutations in non-syndromic hearing impairment in Khuzestan province of the Iran

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Background and aime: Hearing impairment (HI) is one of the most prevalent sensorineural handicaps that occurs 1 in 1000 at new born. More than 50 nuclear genes have been shown to be involved in nonsyndromic HI, but mitochondrial mutations might also cause HI.Mitochondrial mutations are present in 0-2% hereditary hearing impairment .In the present study, we investigated three common mutation of the mtDNA molecule among 62 subject with non syndromic hearing impairment in Khuzestan province of the Iran.

Method: Individual's DNA was extracted using standard phenole-chloroform method. We screened known mtDNA mutations (A1555G, A3243G and A7445G) by PCR-RFLP procedure. Finally, the mutations were found confirmed by direct sequencing.
Results: A1555G and A3243G mutations were not found in our study. However, mutations screening by PCR-RFLP technique and direct sequencing revealed a known A7445C mutation and an unknown G3316A mutation in two hearing impaired patients with frequency of 3.2%.**Conclusion:** we concluded that contribution of mitochondrial mutations in hearing impaired patients in Khuzestan province can be importantly. Then testing for mutation in mtDNA should be considered only when other frequent deafness causing mutations have been excluded.

J02.06
Hereditary hearing loss and consanguinity

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Many of the patients which referred to the departments of otolaryngology are suffering from some sort of hearing loss. Hearing problem usually place stress upon interpersonal relationships, social isolation, and even sadness and depression.The most of the hearing loss is hereditary and follow autosomal recessive pattern of inheritance. Therefore, the frequency of hearing loss is more among those populations which consanguineous marriage is an old tradition.
The aim of this study was to find out the frequency of hearing loss among the consanguineous marriages.
We investigated all the pedigrees of patients which referred to our Department for genetic counseling during the last three years. We found 206 pedigrees with some sort of otolaryngology disorders, and out of them 157 pedigrees were with consanguineous marriages. Among these 157 pedigrees, 496 cases were the result of consanguineous marriages, and 219 patients were affected with otolaryngology disorders, Therefore, these 219 cases were studied according to consanguineous and non-consanguineous marriages, kind and degree of relationship of their parents, patterns of inheritance, and sex, by using SPSS software. Out of 496 cases, 115 patients (23.2%) revealed total deafness, and 53 patients (10.7%) exhibited partial hearing loss.
Our results showed that, on the whole, there is a relationship between hearing loss and consanguinity, and the most affected individuals have consanguineous parents.

J02.07
Investigation of thyroid Transcription Factor-1 and TSH Receptor genes variant in Iranian children with congenital hypothyroidism

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Congenital hypothyroidism(CH) is a metabolic disorder which occurs in 1:3000 or 1:4000 live births. The patients may suffer from thyroid gland dysgenesis or dyshormongensis. Thyroid dysgenesis (TD) may be caused by mutations in Thyroid Transcription Factor1 (TTF-1) or TSH Receptor (TSHR) genes. This study was aimed to analyze TTF-1 and TSHR (exons 5 and 7) genes in Iranian CH patients. Blood samples were collected from the 30 patients and family members in EDTA supplemented vacutainers. Informed consent was obtained from parents or guardian of patients. Genomic DNA from each individual was extracted using a QIAamp Blood Kit (QIAGEN) according to the manufacturer's instructions. The final concentration of extracted DNA was adjusted to a 50 ng/µl to develop the assay. Mutations in TTF1 and TSHR genes were investigated by PCR-SSCP followed by direct sequencing. In this presentation our data regarding TTF1 and TSHR genes mutations screening will be presented and the correlation between each mutation and clinical manifestation will be discussed.

J02.08
A case of Noonan syndrome

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Background: Noonan syndrome (NS) is a sporadic or inherited developmental disorder with an incidence of one in 1.000-2.500 children. Clinical manifestations of NS include a typical dysmorphic appearance, congenital heart defects, short stature, skeletal malformations like scoliosis and chest malformations, cryptorchidism, eye abnormalities and various degrees of cognitive deficits. NS is genetically heterogeneous, recent discoveries revealed a disorder of upregulated RAS-MAPK signaling. Case report: A 12 years old male child was admitted to the Department of Pediatric Surgery of our hospital for multiple musculoskeletal deformities: severe scoliosis with a 400 Cobb angle, funnel chest, bilateral flat feet, joint laxity, and curved thumbs. In addition the child had bilateral cryptorchidism, micrognathia, astigmatism and mental retardation. A management strategy was developed for each type of pathological dysfunctions: for scoliosis we preferred brace correction using Cheneau brace; for cryptorchidism a surgical intervention was scheduled; for astigmatism optical correction was recommended, for mental retardation intervention programs and individualized education strategies were proposed and for short stature therapy with growth hormone was consider. The opportunity of intervention for other problems like: the deformity of the chest, foot and thumbs is still in debate, a balance between risks and benefits being considered. Conclusion: Multidisciplinary treatment is the key to success in managing children with Noonan syndrome and the pediatric surgeon play an important position to lead the health team.

J02.09
Diagnostic and therapeutic management of a case with myelomeningocele in the context of a complex plurimalformative syndrome

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Myelomeningocele is a complex congenital spinal cord anomaly (the most severe form of spina bifida, localized mainly thoracic-lumbar 85%, also thoracic 10%, cervical 5%) that causes various degrees of myelodisplasia. In the context of plurimalformative syndrome, it is most commonly associated with hydrocephaly. The diagnosis is set clinic and imagistic.
Aim: presentation of diagnostic and therapeutic management of a case with thoracic-lumbar myelomeninocele with closed medullar area, in plurimalformative circumstances.
Material and method: we present the case of a newborn, gestational age 37 weeks, birth weight 2000g, APGAR 8, cranial presentation, from an unmonitored pregnancy, pregnant 1 para 1, with cytomegalovirus infection, prolonged jaundice, anemia, admitted in our service 4 days after birth. Clinic: spina bifida with exulceration at thoracic-lumbar level, flaccid paraplegia, bilateral varus equin, short neck, cranial perimeter in evolution.
Results and discussions: Due to malformative associations interdisciplinary exams were performed: ophthalmic, cardiologic (atrial septal defect), neurosurgical, transfontanellar ultrasound (dilated lateral ventricles, absence of corpus callosum), MRI for cervical, thoracic, lumbar spine and head (widening of lateral ventricles, absence of septum pellucidum, lumbar-sacral spine rotated in axis and right lateral, lumbar disraphia with myelomeningocele 1/3.7cm, malrotated kidneys), There were no modifications of cariotype.
Conclusions: 1. Due to malformative association interdisciplinary team cooperation was important especially for ventricle - peritoneal drainage and surgical cure of mielomeningocele. 2. There were no modifications of cariotype, the only modification was the cytomegalovirus infection. 3. Genetic consult and antenatal diagnosis can lead to a favorable prognostic of mielomeningocele and minimal further complications.

J02.10
A woman with 45,X/46,XY karyotype and clinical features of Turner syndrome

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45,X/46,XY mosaics have a wide spectrum of phenotypic appearances such as female or ambiguous external genitalia with bilateral streak gonads or asymmetrical gonadal differentiation. They also have an increased risk of developing gonadoblastoma, and a minority of them are masculinized.Structural X chromosome abnormalities are thought to occur as a result of breakages in the X chromosome with subsequent reunion of X chromosome sequences.

Here we report a 31-year-old woman referred to our laboratory due to infertility. The patient has irregular menstrual cycle. There was a familial history of infertility in her family. Chromosome analysis revealed mos45,X[22]/46,XY[8] karyotype. The clinical features of this case will be discussed and compared with the published cases.

P03 Cytogenetics

P03.001
Intra-familial variable expression of a recurrent 16p13.11 deletion: a severe phenotype associated with a second genomic event.

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Genomic disorders are recurrent microrearrangement syndromes that arise through non-allelic homologous recombination mediated by segmental duplications (SD). Over 20 novel genomic disorders have been described in recent years. Recurrent microdeletions at 16p13.11 span 1.5 - 3 Mb and are located between three sets of SD-associated breakpoints (BP1-BP2-BP3). There are no clear phenotypic differences between BP1 - BP2 and BP1 - BP3 deletions. The 16p13.11 deletion is associated with a broad spectrum of neurocognitive diseases, including autism, intellectual disability, epilepsy and schizophrenia, as well as skeletal and cardiac malformations. It is characterized by a high phenotypic variability, with some carriers having a normal phenotype.

Here we report a family with a father and two daughters carrying a 16p13.11 deletion (BP1-BP3), as determined by Affymetrix 6.0 SNP array. The oldest daughter has speech delay. The youngest daughter presented at birth with failure to thrive, congenital heart defect and features of oto-palato-digital syndrome. In addition to the 16p13.11 deletion, she carries a 7.5 Mb deletion at 10q22.3-q23.2. This deletion is a recurrent, SD-associated, genomic disorder, which has been previously reported in 7 unrelated patients with developmental delay and congenital abnormalities.

This is the first report of coexistence of a 16p13.11 deletion with an additional imbalance, causing a severe phenotype. Additional large imbalances have been previously observed in 25% of patients with 16p11.2 and 16p12.1 deletions, and were proposed to be responsible for the phenotypic variability of 16p rearrangements. Interestingly, the additional imbalance at 10q22.3-23.2 in our patient is also a recurrent genomic disorder.

P03.002
Genomic rearrangement dup(18p)/del(18q) studied by SNP-array, MLPA and BAC-FISH techniques

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Partial duplication-deletion of the short and long arms of chromosome 18 has been rarely reported in literature with few cases including molecular cytogenetic studies. We describe a 18-month-old boy with severe growth deficiency pre-and post-natal, microcephaly, dysmorphic features, epilepsy and intense hypotonia. The 18q deletion was first detected by G-banding karyotype and appeared to be a small deletion. Molecular techniques, including whole genomic array, multiplex-ligation probe amplification (MLPA) and fluorescence in situ hybridization (FISH) with BAC probes were required in order to better define the rearrangement, to determine the breakpoints and to better delineate the genotype/phenotype correlation. The Cytogenetics Whole-Genome 2.7M Array (Affymetrix) showed a result arr 18p11.3 2p11.22(234,125-9,782,925)x3, 18q21.2 q23(50,293,625-76,084,580) x1 dn. Thus, the patient presented a 9.6 Mb duplication, from 18p11.32 to 18p11.22, and a 25.8 Mb deletion, from 18q21.2 to 18q23. MLPA with subtelomeric probes revealed three copies of 18 psubtel and one copy of 18 qsubtel. FISH technique using RP11-113J12 and RP11-231N16 probes confirmed the results, since these probes were in upside down order. Thus, the duplication/deletion rearrangement probably originated due to an abnormal meiotic recombination within a pericentric inversion. The deleted and duplicated regions encompass genes whose imbalances contributed to the severe phenotype in the patient. These data highlight the fact that concomitant deletions associated with inverted duplications are more frequent than classical cytogenetic methods alone have been able to identify. Furthermore, our results show the usefulness of high-resolution molecular cytogenetic analysis both in the delineation of chromosome rearrangements and in clinical diagnosis.

P03.003
Familial 1q21.1 microduplication associated with cardiomyopathy and absent pulmonary valve

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Introduction: The chromosome region 1q21.1 contains several low-copy repeats which makes this region susceptible for cytogenetic rearrangements. These rearrangements are associated with abnormal head size, mental retardation, autism and congenital anomalies. Most often, microdeletions and -duplications of 1q21.1 occur *de novo*. We describe a large family with a 1q21.1 microduplication.

Methods & Results: The proband is the first child of non-consanguineous Dutch parents. Prenatal ultrasound revealed an enlarged heart with a ventricular septal defect and wide aortic and pulmonary arteries. After pregnancy termination, external morphologic examination demonstrated a proportioned fetus with a round face, hypertelorism and short palpebral fissures. Autopsy showed a grossly enlarged heart, perimembranous ventricular septal defect, absent pulmonary valve, dysplastic tricuspid valve, persistent left superior vena cava and abnormally wide and thickened wall of the ascending aorta. Genome wide SNP array analysis on DNA from cultured amniocytes revealed a 1q21.1 microduplication of 2.2 Mb. This microduplication was also found in the mother, who presented with mild mental retardation. Subsequently, this rearrangement was detected in 9 family members. All displayed dysmorphic features including hypertelorism and macrocephaly. Normal development was present in most 1q21.1 microduplication carriers.

Discussion: Congenital heart malformations have been reported in 4/26 patients with 1q21 duplication from two large cohorts [Mefford 2008, Brunetti-Pierri 2008], including ventricular septal defects and univentricular heart. *GJA5* and *PRKAB2* are good candidate genes for the cardiac defects observed in these patients.

Conclusion: Our description of this unique 3-generation family underlines the incomplete penetrance and extreme phenotypic variability of 1q21.1 microduplications.

P03.004
Congenital heart defects in a novel recurrent 22q11.2 deletion syndrome harboring the genes *CRKL* and *MAPK1*

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The proximal region of the long arm of chromosome 22 contains eight low copy repeats (LCR), known as LCR22s. Non-allelic homologous recombination (NAHR) between these recombination substrates explains the existence of recurrent rearrangements within the 22q11.2 region, and the high prevalence of *de novo* events. The most common recombination event occurs between LCR22-2 and LCR22-4, and gives rise to a 3.0 Mb deletion, associated with DiGeorge syndrome.

We report on a novel recurrent 22q11.2 deletion syndrome in a 14-months-old girl with a common arterial trunk, growth delay and mild dysmorphic features. She presented a normal development. The current deletion spans LCR22-4, the distal part of the common 22q11.2 deletion syndrome and the proximal part of the distal 22q11 deletion syndrome. This deletion did not occur by NAHR between any of the major LCR22s. However, by means of extended long template PCR the proximal and distal breakpoint containing regions were found to coincide with a 3.7 kb block of 97% homology between 19,351,886-19,355,637 and 20,799,044-20,802,748. We believe that these homologous regions served as recombination substrates for this novel 22q11.2 deletion.

An identical deletion was described recently in a normally developing girl with imperforate anus and multiple septal defects (Ogilvie *et al.,* 2009). Interestingly, both cases presented with a congenital heart defect (CHD) and growth delay, but no developmental delay. Automated gene prioritization, based on ENDEAVOUR technology (Aerts *et al.,* 2006), was applied to identify candidate genes for CHD within this region. The highest ranking genes were *CRKL* and *MAPK1*.

P03.005
Molecular cytogenetic characterization in a child with 2q deletion and 4q duplication

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Many patients referred for psychomotor retardation (PMR), dysmorphic features and/or congenital anomalies are predicted to have cytogenetic imbalances, which can not be identified by conventional methods. We present here genetic study on an 18 years old male investigated for severe mental retardation associated with dismorphic features, limb anomalies, cadiac and renal malformations. Conventional cytogenetics: abnormal chromosome 2, with additional material on the long arm, add(2q). The banding pattern of abnormal chromosome suggested that the additional segment 2q belongs to telomeric short arm, or telomeric long arm of chromosome 4. WCP4 probe was used and hybridized to the additional region of derivative chromosome 2. For differentiation, in addition a telomeric 4p probe was used, which did not hybridise to the segment attached to the long arm of the derivative chromosome 2. We concluded that the additional material of chromosome 2 belongs to chromosome 4. To delineate correctly the region implicated in the chromosomal rearrangement aCGH analysis (105A Oligo Microarray Kit) was performed and revealed a 7.5 Mb deletion on 2q37.3 and a 29 Mb duplication on 4q32-4q35.2. We compared the clinical features of our patient with other cases described in the literature and we found more than 60 cases of 2q37 terminal deletion reported with features ranging from developmental delay, mental retardation, dysmorphism, autism, cardiac or renal abnormalities. The chromosomal bands 4q32-q35 was associated with PMR, axial hypotonia, multiple congenital anomalies and hand/foot malformation. The aCGH analysis is a useful method for the detection and characterization of chromosomal rearrangements.

P03.006
2q34-qter duplication in a patient with developmental delay

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Patients with 2q3 duplication syndrome show variable developmental delay, facial and visceral anomalies. The 2q34-qter duplication is apparently crucial in determining the phenotype, as shown by seven previously reported cases of 2q34-qter duplications.

We present a 10 months boy with developmental delay, growth retardation, hearing problem, facial and non-facial minor anomalies. He was born to a 30 years old mother with a history of recurrent fertility problems. She went through 3 spontaneous abortions, 2 of her children died at 8 days and 4 months as a result of water loss or hypotonia and cleft palate, respectively, and now she has one healthy boy. Karyotype analysis of the proband showed an additional segment of unknown origin on 4q35. Karyotyping of both parents showed that the mother carries a balanced translocation t(2;4)(q34;q35) and identified 2q34-qter as the origin of the additional segment.

The proband has a round face with high forehead, broad nasal bridge, high arched palate, macrostomy, long philtrum, thin upper lip, ear pit and flat occiput, confirming the importance of the distal region of HSA2 in the 2q3 duplication phenotype. Other findings include shawl scrotum with undescendent and hypoplastic testes, bilateral simian creases, clinodactyly with short fifth finger, hyperflexible joints, hypotonia, growth retardation, slow developmental motor function, and mild ventriculomegally in brain CT scan. Notably, none of the major congenital anomalies, such as congenital heart disease or urinary tract malformations found in more proximal duplications, are present suggesting a critical region from band 2q31 to q34.

P03.007
5q35.2 → ter deletion in a patient with craniofacial feature of sotos syndrome and growth retardation

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Sotos Syndrome (SoS) is a congenital disorder characterized by excessive growth, typical craniofacial features, and various degrees of developmental retardation. Mutation of NSD1 (nuclear receptor SET-domain-containing protein) located at 5q35.3 are responsible for most cases. We present a 2 1/2-year-old girl in this study with unusual phenotype including macrocephaly, prominent and wide forehead, coarse face, hypertelorism, wide mouth, prominent jaw, premature eruption of teeth, low-set ears, hypotonia, hallux valgus, wrinkled skin, hearing loss, developmental delay and growth retardation (height and weight below the 3rd percentile) . Based on the clinical findings chromosomal study and Multiple Ligation Dependent Probe Amplification (MLPA) was requested with suspicion of Sotos syndrome. MLPA showed microdeletion at q35.3 which is associated with Sotos syndrome. Karyotyping confirmed deletion of long arm of chromosome 5 (q35.2 and the segment distal to this band. Since our patient had additional findings and differences compared to Sotos syndrome namely coarse features, wrinkled skin, hyperextensibility of joints, mainly in digits, growth retardation, while Sotos patients have excessive growth (>97th centile) array comparative genomic hybridization (aCGH) was performed showing a 0.5 Mb deletion in 5q35.2◊ ter with maximum,174416712 and minimum,174837769 breakpoint boundaries. The additional findings are most probably related to deletion of adjacent genes to NSD1.

P03.008
Characterization of a case with an atypical deletion 8p23.1

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Distal deletions of chromosome 8p are associated with congenital heart defects. Other features include microcephaly, mental retardation and facial anomalies. *GATA4* has been involved in the heart defects and, the *TNKS* in the behavioural problems and learning disabilities. The region between them, including *MSRA* and *SOX7*, has been suggested to play a critical role in microcephaly and facial dysmorphism.
Some cases share a commonly deleted region of ~6Mb with breakpoints clustering in the same regions (low copy repeats 8p-OR-REPD and 8p-OR-REPP). However, there are exceptional cases with larger or smaller deletions with variable phenotype so, it is necessary to investigate the precise deleted region in atypical cases, to define the minimal critical region.
We report a 3 year-old non dysmorphic girl with microcephaly, speech and developmental delay and normal cardiac evaluation. Karyotype showed a *de novo* del(8)(p23). FISH and MLPA confirmed a terminal deletion between 10.2 and 11 Mb involving *TNKS* and *MSRA*, but not including the GATA4 region (TelVysion 8 Vysis, P036 telomere, P096 MR2 and P311 congenital heart disease, MRC-Holland).
Although the patient presents a larger deletion than previously reported, she presents less features, therefore it is important to test which specific genes are deleted. On one hand, *GATA4* was not deleted as we expected. On the other hand and as it has been proposed, *MSRA* deletion could explain microcephaly in our case but other specific molecular analysis (aCGH) will be performed to find out if *SOX7* is present, explaining the absence of facial dysmorphism.

P03.009
Molecular cytogenetic analysis of 8p23.1 deletion associated with moderate mental retardation without heart defect

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To date more than 50 cases of interstitial or terminal 8p23.1 deletions have been reported. This aberration is especially prone to various genomic rearrangements mainly because of the existence of the two olfactory receptor gene clusters (REPD and REPP) which is associated with a spectrum of anomalies that can include congenital heart malformations and congenital diaphragmatic hernia (CDH).
We report on a 5-year-old boy with psychomotor delay, moderate mental retardation, hypotony, vertebral anomalies, and dysmorphic features without heart malformations nor any overlapping features of Cornelia de Lange syndrome (CdLS).
Using Array comparative genomic hybridization (CGH array) on a 44K resolution, we identified an interstitial deletion of 1,181,530 pb in 8p23.1: arr 8p23.1 (8,229,404 - 9,410,934) X1. Fluorescence in situ hybridization (FISH) analysis confirmed the deletion using RP11-429B7 probe.
The mental retardation presented by our case seems to be associated to genes haploinsuffiency included in the 8p deletion. This deletion includes TNKS gene, known as a candidate gene for CdLS, supporting other studies invalidating TNKS in CdLS. In addition, the absence of cardiac malformations often associated with the 8p deletions could be explained by the retention of SOX7 and GATA4 genes, known for their major role in cardiac development. Even though, further studies based on the high-resolution characterization of additional cases are needed to establish more clearly the role of the discussed candidate genes in carriers of 8p23.1 deletions.

P03.010
Molecular characterization of a complex rearrangement associated with an apparently balanced translocation

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We report on a 42 year-old male with moderate mental retardation, facial dysmorphic features and skeletal anomalies, carrier of a complex chromosome rearrangement, consisting of a reciprocal translocation plus a deletion detected by conventional karyotype: 46,XY,t(3;13)(q26.2;q22),del(6)(q15q21). FISH analysis with subtelomeric probes confirmed the translocation. Further studies with array-CGH analysis (Agilent, 180K) demonstrated the presence of several copy number variants, including a duplication on chromosome 3, two deletions on chromosome 6, and a deletion on chromosome 13: arr 3q26.1(163,992,703-164,108,151) x3, 6q13q14.1(75,314,285-76,187,164)x1, 6q14.1(80,785,392-80,911,216)x1, 13q31.3(89,597,407-90,636,021)x1.
Deleted regions on chromosome 6 were 873Kb and 126Kb in size, and were separated by a 4.6Mb normal region. The deletion included 4 genes, one of which (COL12A1) is a known candidate gene for connective tissue pathology and is probably involved in the kyphosis and foot deformities seen in our patient.
The small size of the 6q deletion (~1Mb) and the presence of copy number variations on both chromosomes 3 and 13, suggest the existence of a more complex rearrangement than the one previously described. Therefore additional analyses should be done to unravel the nature of this rearrangement.
This study provides further evidence that cryptic genomic imbalances are common in patients with apparently balanced translocations and abnormal phenotypes, and emphasizes the importance of using high resolution genome-wide analysis to characterize complex rearrangements.

P03.011
A 2p16.1 deletion in a boy with MR/MCA syndrome detected by MLPA

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Chromosomal abnormalities are a common cause of mental retardation / multiple congenital anomalies. The advent of new technologies for molecular cytogenetics such as FISH, MLPA, array CGH has dramatically increased the number of novel microdeletions and microduplications associated with a wide range of clinical phenotypes of a relative stable and distinctive MCA/MR syndromes.
We report a 5 months old boy with developmental delay, failure to thrive, severe hypotonia, hypoplastic genitalia (micropenis, cryptorchism), syndactily 4-5 of the left hand and facial dysmorphic features, including brachycephaly, strabismus, short and narrow palpebral fissures.
Routine cytogenetic studies at amniocentesis and postnatally (300-400 band level) were normal.
MLPA, performed using kit P245 (MRC Holland), detected deletion of the loci FANCL, 2p16.1 and REL, 2p16.1 and follow-up studies by arrayCGH were performed.Despite the early age our patient presents some of the characteristic feature of the 2p15-16.1 microdeletion syndrome. The clinical follow up of the patient and the determination of the deletion size by additional molecular techniques (array CGH, FISH etc.) will contribute to the further delineation of the syndrome phenotype and the underlying genes since a few cases are described up to now.

P03.012
A *de novo* 1.8 Mb 17q21.33 microdeletion detected by SNP-CGH in patient with mental retardation and dysmorphic features

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We report 16 years old male presenting with mild mental retardation and dysmorphic features (slight shortness of stature, poor weight gain, long face, beaked nose, thick lower lip, micrognathia, malocclusion of teeth and clinodactyly of the 5th fingers). The boy was born to healthy, non-consanguineous Lithuanian couple after a normal pregnancy in a normal delivery. His older half brother was healthy. The proband had a normal motor development, but language development was severly retarded. Karyotyping of peripheral blood cells revealed an inconspicuous karyotype. Analysis of patient genomic DNA by Illumina whole-genome genotyping (Infinium HD) assay using HumanCytoSNP-12 BeadChip revealed an interstition 1.8 Mb deletion of 17q21.33. FISH analysis

of patient and both parents confirmed the microdeletion as *de novo*. Among 23 genes in deleted region, haploinsufficiency of *CACNA1G* and *CA10* genes can play crucial role in developmental delay. Both of genes are involved in brain development and neurological processes. The *CACNA1G* gene, which predominantly is expressed in brain, encodes voltage-dependent calcium channels which are thought to be involved in neuronal oscillations and resonance, pacemaking activity in central neurons, neurotransmission. The *CA10* gene encodes the protein which is acatalytic member of the alfa-carbonic anhydrase subgroup and is thought to play significant role in the central nervous system, especially in brain development. Based on this information the reported *de novo* deleted region is considered as pathogenic.
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P03.013
Recurrence of a 19p13 duplication by a familial intrachromosomal insertional translocation

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Introduction: the occurrence of pure 19p13 duplication in two first grade cousins led us to investigate the presence of an insertional translocation. Intrachromosomal insertional translocation was found in at least three generations. Three mentally retarded cases and multiple abortions in the family were found.

Material and methods: whole genome array-CGH was performed on Agilent oligo-chip 44K. Microsatellite markers and MLPA were used to confirm the array-CGH findings. FISH using BAC probes were used to locate and confirm the insertional translocation.

Results: in a series of 200 patients with mental retardation associated with congenital anomalies, we found one patient with an interstitial duplication in 19p13. Initially it was thought that this change had arisen de novo because the duplication was not present in the parents of the index case. However, the appearance of another case with the same duplication in the family made us suspect the presence of an insertional translocation. This was confirmed by FISH with region-specific probes.

Discussion: Insertional translocations can be much more common than initially thought. Therefore, following the detection of a pure duplication or deletion that causes disease in a patient, it should be characterized by FISH in order to offer adequate genetic counselling.

P03.014
De novo deletion 10p14 in patient with mental retardation, speech impairment and hypothyroidism

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The use of array CGH for clinical testing of patients with mental retardation/ developmental delay and/or congenital anomalies has provided a possibility to identify novel recognizable microdeletion/ microduplication syndromes, as well as to expand the phenotype. Additional report of smaller chromosomal rearrangements, allow to establish more accurate phenotype-genotype correlation or single genes impact to the phenotype. Microdeletion of 10p14-15 is characterized by HDR or DGS2 syndromes. We report on 11years old boy carrying 2.77 Mb interstitial deletion at 10p14, with mild mental retardation, speech impairment, hypotonia, hypoplastic corpus callosum, hypothyroidism with good respond to levothyroxin therapy, hypermetropia, syndactyly, dysmorphic features (narrow forehead, round face, simple ears, small nose). 2.77 Mb deletion was detected by Agilent 105K oligo-array genome hybridization and involves the genomic region between 8,255,605 and 11,023,139 base pairs on chromosome 10 (NCBI build 37.2). This deletion is one of the smallest from previously reported, involving only two genes (*SFTA1P1* and *LOC254312)*. We do not observe symptoms of HDR or DGS2 syndromes. Besides MR and speech impairment (characteristic for other patients with deletion in 10p15), our case is associated with few distinct features, as brain structure abnormalities and thyroid hypofunction. As adjacent region (10p15) contains many genes and is related with MR and speech impairment, the possibility of disruption of regulatory sequences by downstream deletion should be considered.
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P03.015
Molecular cytogenetic studies of 1p36 deletion syndrome in children with mental retardation and congenital malformations

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Deletions of 1p36 are associated with a common microdeletion syndrome affecting one in 5000 live borns. Diagnosis of 1p36 deletion represents a challenge for cytogeneticists inasmuch as these deletions are hardly visible by classical cytogenetic techniques. The solution is the application of FISH or array CGH. However, the former shows only loss of a small chromosomal region, whereas the latter is expensive for numerous laboratories around the world. To avoid significant expenses, we have tested whether high-resolution CGH (HR-CGH) can be used for diagnosis of 1p36 deletion syndrome. Cytogenetic studies of 3500 children with mental retardation and congenital malformations allowed to identify the deletion in one case only. Additional analysis of 100 cases non-randomly selected according to phenotypical manifestations by HR-CGH provided for detection of three other 1p36 deletions: del(1)(p36.1p36.3), del(1)(p36.13p36.21), del(1)(p36.22p36.23). Array CGH analysis has confirmed data obtained. Deletion sizes varied from 4.4 Mb to 12 Mb and encompassed regions previously reported to be involved in 1p36 deletion syndrome. It is noteworthy that one case demonstrated multiple chromosome abnormalities: constitutional 47,XXY and mosaic 12p tetrasomy in addition to 1p36 deletion. Phenotypic manifestations were similar to those usually observed in 1p36 deletions, being, however, insufficient for the clinical diagnosis of the syndrome. Our data shows that HR-CGH can be used for diagnosis of 1p36 deletion syndrome (prescreening before array-CGH application). Finally, we have demonstrated 1p36 deletion to occur as frequent as 1/875 in a clinical population of children with mental retardation and congenital malformations inhabiting Central Russia.

P03.016
Overlapping microdeletions in 15q22.1-q22.2 in two patients with intellectual disability - characterization of the critical region using the DECIPHER database

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Here, we report two patients with intellectual disability (ID) and de novo microdeletions in 15q22.1-q22.2 detected by molecular karyotyping. The smallest region of overlap (SRO) of the two microdeletions contained 26 genes, 8 of which are listed in OMIM.

Patient 1, a 14 1/2 year old boy presented with moderate ID, slightly disproportional growth retardation, truncal obesity, strabismus and mild facial dysmorphisms. His IQ at the age of 6 8/12 years was 55-60. After an initial speech delay, his language skills at the age of 14 1/2 years were significantly better in comparison to his IQ.

Patient 2, a 6 1/2 year old male, was identified in the DECIPHER database. He presented with mild global developmental retardation, muscular hypotonia, uncoordinated movements, joint hypermobility, and mild dysmorphisms.

Molecular karyotyping detected de novo microdeletions in 15q22.1-q22.2 of 5.3 Mb and 5.5 Mb with an SRO of 4.97 Mb and 8 OMIM genes. However, the critical region could be reduced tentatively by the deletion in a third DECIPHER patient without ID, obesity, muscular hypotonia or dysmorphisms. His deletion overlaps the proximal 1.87 Mb of the SRO of patient 1 and 2, narrowing down the critical region to 3.09 Mb and 11 genes. Only two of the three remaining

OMIM genes are promising candidate genes for ID: VPS13C which is highly expressed in the central nervous system and RORA (retinoic acid receptor) which is involved in neuronal development and may also cause the muscular phenotype of patient 2 since it interacts with MYOD.

P03.017
SNP-array analysis in 64 Polish patients with intellectual disability - first results of the CHERISH project

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The CHERISH consortium was established in 2009 with the main goal of developping a standardized approach to genetic tests in patients with intellectual disability (ID) in Eastern European and Central Asiatic countries.

In recent years, 239 Polish families of well clinically characterized patients with idiopathic ID have been included in the CHERISH study. Preliminary analysis (karyotype, *FMR1* molecular test, metabolic investigation) was followed by MLPA screening for microdeletions/microduplications and subtelomeric rearrangements. In patients with normal results or with complex chromosomal rearrangements search for cryptic chromosome rearrangements was carried out through array-CGH or SNP array analysis in partner laboratories in Bologna and Tartu.

The authors present results of SNP array in 64 investigated individuals. The size of structural aberrations with potential clinical significance found in 10 out of 64 investigated individuals varied from 800 Kb to 23 Mb.

These data demonstrate that SNP array analysis is necessary to tie complex karyotypes to phenotypes of patients with ID and that cooperation on the European level is crucial to achieve diagnostic standards in ID.

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P03.018
A patient with a 7 Mb de novo 2p12p13.3 deletion associated to cerebral anomalies, muscular hypotonia and dysmorphisms

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We describe a patient with moderate mental retardation, atrial septal defect type II, epilepsy and peculiar facial features. Brain MRI revealed hypoplasia of the cerebellar vermis and widespread dilatation of cerebral sulci and fissures. The neurological examination revealed hypotonia, ataxia and dysmetria.

Array-CGH with an average resolution of about 75-100 Kb showed a 7 Mb de novo 2p12p13.3 deletion. The rearrangement includes 75 genes, 10 of which are known disease gene. Five patients with del2p11.2p13, detected with traditional cytogenetic analysis, have been reported in literature and overlapping with the deletion reported. The deletion present in our case also overlaps with three cases reported in DECIPHER database and identified with molecular cytogenetic analysis: a case with a smaller deletion, 780 kb, in region 2p13.3p13.2 and two cases with wider duplication, a 25.17 Mb duplication in region 2p16.2p12 and a 12.13 Mb duplication in region 2p13.3p12.

We discuss the phenotype of the patient, the overlap with other cases reported to date and the possible genotype-phenotype correlation.

P03.019
Array genomic hybridization characterization of an interstitial 2q36 deletion

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Interstitial 2q36 deletion is a rare event. Less than 10 patients have been reported with interstitial deletions involving this region. Patients carrying this deletion exhibit mental retardation usually severe. This aberration involves at least three genes known for their role in the developing nervous system.

We report here, the case of a 9 years old boy from non consanguineous couple and with familial severe mental retardation, language delay, epilepsy and mild facial dysmorphism. Conventional cytogenetic analysis was performed on the peripheral blood lymphocytes at 450-550 RHG banding showed a little asymmetry on the terminal chromosome 2q. Using array comparative genomic hybridization (CGH array Agilent 44K), we identified an interstitial deletion of 2(q36.3-q37.2) of 8,06Mb: arr2q36.3 (227,508,858- 235,569,556)X1.

This chromosomal aberration carries at least 24 genes, 3 genes are mainly involved in mental retardation: SLC19A3 (Solute Carrier family 19 member 3), DNER (Delta and Notch-like EGF-related receptor) and TRIP12 (Thyroid hormone receptor- interacting 12). All are involving in the nervous system developing. Besides mental retardation, other clinical disorders such as renal cysts, congenital abnormalities could be associated to this anomaly but this phenotypic spectrum depends on the deletion size and so the deleted genes. Therefore, further studies are needed to establish more clearly the effect of 2q deletion especially on the mental status

P03.020
Mendelian molecular cytogenetics in familial microcephaly: the Leuven's experience

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Microcephaly is an important neurologic sign and represents a frequent reason for reference to neuropediatric and neurogenetic clinics. Microcephaly is usually defined as an occipitofrontal circumference (OFC) more than 2 standard deviations (SDs) below the mean for age and gender. Microcephaly may result from any event disturbing the initial stages of brain development and can be associated with more than 450 syndromes listed in the London Dysmorphology Database. Coexistent neurologic disorders include intellectual disability (50%), epilepsy (40%), ophthalmologic manifestations (20%-50%) and cerebral palsy (20%). A genetic etiology is found in 15,5% to 53,3%.

We applied high-resolution molecular cytogenetics using BAC and oligonucleotide-based array comparative genomic hybridization (aCGH) (successively the 1 Mb resolution BAC aCGH and the 32k BAC aCGH (The Sanger Institute-UK) and the OGT CytoSure ISCA 180k DNA oligoarray set (OGT, UK)) to investigate the genetic bases of microcephaly in familial cases referred between 2007 and 2010, to the genetics and pediatric neurology clinics of the University of Leuven for developmental delay and multiple congenital anomalies.

We identified 2 novel loci for microcephaly in 2 families investigated for primary microcephaly and intellectual disability associated with, respectively, a 3,7 Mb del(1q43) and a 6,25 Mb dup(4q13). Both were inherited from affected mothers and maternal grand-mothers. In addition, we mapped two familial microcephalies to the boundaries of the 16p11 (180 kb) and 16p12 loci (670 kb). Candidate genes are prioritized and genotype-phenotype correlations will be discussed together with the genetic counseling in these heterogeneous and challenging clinical conditions.

P03.021
Array CGH detection of genomic/chromosomal rearrangements in children with mental retardation and congenital malformations: the first Russian experience

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Array CGH has become one of the most popular molecular cytogenetic techniques for diagnosing genomic and chromosomal disorders. However, this powerful approach has never been systematically applied to Russian cohorts of children with mental retardation and congenital malformations. Here, we present an array CGH study of 30 children with mental retardation and congenital malformations non-randomly selected according to clinical and cytogenetic data. We were able to detect genomic/chromosomal rearrangements in 14 cases (47%). These were additional chromosome X with 1p36 deletion and mosaic isochromosome 12p; del(3)(p21.32p21.33); del(4)(p16.1pter)/dup(8)(p23.1pter); del(7)(q36.2qter); del(9)(q34.2q34.3)/del(21)(q22.3q22.3); del(10)(q26.2q26.3); dup(10)(q25.2qter)/del(13)(q33.3qter); r(11)(p15.5q24.1)(loss of 11q24.1qter); dup(19)(p13.3pter); del(19)(q13.12q13.13); del(20)(q11.21q11.21); del(X)(p22.2pter)/dup(3)(p22.3pter); dup(X)(q28qter); del(Y)(q11.223q11.223). Size of the rearrangements varied from 1.2Mb to 36.3Mb. Clinically relevant CNVs were detected in 9 cases (30%). Pathological value of CNVs was assessed by bioinformatic assays using gene expression and proteome (interactome/reactome) meta-analysis by Gene Expression Omnibus, BioGPS, Cytoscape 2.8.0 and Pathway Commons. One case exhibited constitutional genomic instability manifested as multiple deletions and duplications (size: from 1Mb to 2.5Mb). To date, similar cases have not been reported in the available literature. These results generally agree with those of previous array CGH cohort studies, showing, however, previously unreported genomic rearrangements in mental retardation and a higher incidence of clinically relevant CNVs. Moreover, we found the application of bioinformatic assays to be valuable for proper interpretation of array CGH data. Finally, to the best our knowledge, this communication represents the first reported array CGH study of a clinical cohort of children with mental retardation and congenital malformations in Russia.

P03.022
12p13.33 microdeletion syndrome: redefining the associated clinical phenotype

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Subtelomeric rearrangement associated with developemental disabilities detection rate represents 5% of the etiologies. Sub-telomeric or interstitial deletions of less than 5 Mb involving chromosome 12p13.33 is one the least frequently deleted region. Only 5 patients diagnosed with FISH telomere analysis or array-CGH have been reported. The most phenotypic manifestation of this microdeletion seems to be speech delay associated with autism spectrum disorders (ASD) phenotype. Two of them were inherited from an apparently healthy parent, suggesting variable expressivity and incomplete penetrance.

A French collaboration together with a search in the Decipher database permitted to gather 6 novel patients (4 probands) with a 12p13.33 subtelomeric or interstitial rearrangement identified by high resolution array-CGH to further delineate this rare microdeletional syndrome. Speech delay was found in 5/6 patients, with dysarthry in 3/6, mental retardation in 4/6 patients, ASD phenotype in 3/6 patients, mild non-specific dysmorphism (3/6) with prominent ear lobes in 2/6 patients, normal growth parameters with macrocephaly in 1/6 patient and microcephaly in 1/6. Variable expressivity or incomplete penetrance was found in both familial cases. The clinical and molecular data of this group of patients were compared to the other observations of the literature. The ELKS/ERC1 gene is found in the commonly deleted region, and appears as a good candidate for speech delay.

These results reinforce the hypothesis that the 12p13.33 subtelomeric or interstitial deletions can be responsible of a variable phenotyope ranging from normal developpement, speech delay, to mental retardation and ASD.

P03.023
First familial case of partial 3p trisomy and Xp monosomy resulting from a maternal translocation characterized by array CGH

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Partial trisomy of 3p is an uncommon chromosome abnormality usually produced by an unbalanced translocation and characterized by recognizable patterns of malformation. Here, we report on two sisters presenting with mental, growth and psychomotor retardation, seizures, pectus excavatum and square facieses with prominent cheeks who exhibited 46,X,add(X)(?::p?22->qter),16qh- karyotype after G-banding karyotyping. Array CGH analysis with Spectral Chip allowing genome scan at 0.3-1Mb resolution has demonstrated duplication of 3pter->p22.3 spanning 36.3 Mb and deletion of Xpter->Xp22.2 spanning 6.4 Mb resulted from an unbalanced translocation t(X;3). Cytogenetic evaluations of parents have shown a presumably balanced translocation between chromosomes X and 3 in mother. Therefore, partial 3p trisomy and Xp monosomy in the index cases have resulted from a maternal translocation. *In silico* analysis of rearranged chromosomal regions has shown that 3p duplication involves 285 genes (107 genes are indexed in OMIM) whereas Xp deletion involves 50 genes (22 genes are indexed in OMIM). Additionally, copy number variations (CNVs) located on 9q12, 10q11.22, 15q11.2 and 15q26.3 were detected. However, these CNVs are likely to be benign, taking into account data obtained by bioinformatic analysis (meta-analysis of gene expression) and by comparison with previously reported benign CNVs indexed in genome variation databases. Although there are no fewer than 30 reports on similar chromosomal abnormalities, to our knowledge there are no descriptions of array CGH analysis of partial 3p trisomy in the available literature. Therefore, these are the first cases of partial 3p trisomy addressed by high-resolution molecular cytogenetic technique of array CGH.

P03.024
Molecular cytogenetic and bioinformatic studies identify loss of *LIMBR1* and *MNX1* as a cause of lumbosacral dysgenesis: array CGH, FISH and gene expression meta-analysis in two independent unbalanced terminal rearrangements of 7q

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Previously, cytogenetic analyses of terminal 7q imbalances have suggested that loss of 7q36 can lead to lumbar or sacral dysgenesis/agenesis. However, apart from reports on *MNX1* mutations in Currarino syndrome (partial sacrum absence and anorectal anomalies), intrinsic causes of lumbosacral dysgenesis associated with genetic defects within the terminal part of chromosome 7q have not been established. Here, we present molecular cytogenetic and bioinformatic analyses of two cases of terminal 7q loss. The first case is an unbalanced translocation t(7q;21q)(q34;q22.13) reported previously (Vorsanova et al., 2008), whereas the second one is a deletion of 7q36 both featured by characteristic facial dysmorphisms, mental retardation, lumbosacral dysgenesis. Using array CGH and FISH, we have narrowed the region of chromosome 7 - 7q36.2q36.3 (from ~152Mb to ~158Mb) - associated with common phenotypic features in these two cases. To identify genes associated with lumbosacral dysgenesis, we have further studied the deleted region by gene expression meta-analysis using Gene Expression Omnibus and BioGPS. Comparative analysis

of expression profiles of 45 genes located within the aforementioned chromosomal region in fetal and postnatal tissues have indicated that two genes *LIMBR1* and *MNX1* are the most likely candidates for lubosacral dysgenesis. Taking into account that *LIMBR1* is involved in bone morphogenesis and mutations in *MNX1* are likely to lead to sacral agenesis, we have concluded that loss of *LIMBR1* and *MNX1* causes lumbosacral dysgenesis. Finally, array CGH genome screening in combination with gene expression meta-analysis seems to represent a powerful approach for identification of genetic causes of diseases.

P03.025
Molecular karyotyping identified pathogenic CNVs in 3 patients with developmental delay and/or multiple congenital anomalies

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Oligo array-CGH was applied in 3 patients with developmental delay and multiple congenital anomalies in order to unravel the underlying genetic abnormalities. We have used BlueGnome CytoChip oligo 2X105K microarray, v1.1, with 35kbp backbone resolution. All abnormal results were confirmed with fluorescence in situ hybridization (FISH).
In one of the patient with karyotype 46,XY,inv(9)(p11q13) array CGH revealed duplication of (8)(q24.13q24.3) region involving 22764514bp and deletion of (9)(p24.3p22.2) region encompassing 18023616bp. It was initially assumed that the patient's imbalances are de novo, but the subsequent examination of the proband's family and the fine analysis of the FISH results revealed that it comes to familial reciprocal translocation between the chromosome 8 and the inverted chromosome 9. The second patient was with blurred karyotype - 46,XY,add(15q),add(18)(p11.2). Array CGH detected two pathogenic CNVs - dup(18)(p11.22p11.21) spanning 5479842bp and dup(6)(q16.1q21) with 21333134bp involved. The FISH validation highlighted the chromosomal rearrangement: it comes to insertion of the duplicated region of chromosome 6 in the long arm of chromosome 15 and partial trisomy 18p. The karyotype of the third patient was normal - 46,XX. The molecular karyotyping identified deletion of 12978687bp in (6)(q25.3q27) region and duplication in (10)(q25.2q26.3) region involving 21360000bp.
This study proves that array-CGH is a powerful method to identified genomic imbalances not detected at the level of GTG banded karyotype analysis. Furthermore, it shows that for provide correct diagnosis, one molecular cytogenetic method is not enough, although some of them (i.e. array CGH) have high-resolution capability.
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P03.026
Investigation of Subtelomeric Chromosome Abnormalities in 50 patients with Idiopathic Mental Retardation and its comparison with 50 Control Normal Cases

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Mental Retardation (MR) has heterogeneous etiology mostly with genetic causes. It has been shown that 4-12% of idiopathic MR have subtelomeric rearrangements using MLPA technique. In this study, using MLPA technique, the subtelomeric abnormalities were investigated in 50 patients with idiopathic MR with or without dysmorphism, with non consanguineous parents as well as 50 normal people. All the patients had normal karyotypes and were negative for fragile-X. The patients were first screened with one MLPA kit, P036-E1. The abnormal cases were re-checked using a different set of MLPA kit, P070-A2. All the controls were normal for the subtelomeric regions. However, four patients (8%), using both P036 and p070 kits, had abnormal results. In this study, the abnormalities were duplication of subtelomeric region of long arm of chromosome 19, duplication of subtelomeric region of short arm of chromosome X/Y, duplication of subtelomeric region of short arm of chromosome 6/deletion of subtelomeric region of long arm of chromosome 10, and duplication of subtelomeric region of short arm of chromosome 19/deletion of subtelomeric region of long arm of chromosome 22. MLPA studies using both kits were carried out on the parents. Only the case with dup X/Yp was inherited from the mother. The other three patients had normal results for the parents. Therefore these abnormalities are pathogenic. FISH investigation on the parents in order to see whether the abnormalities are inherited or not are pending. Genetic counseling and prenatal diagnosis will be recommended to the affected families.

P03.027
Use of SNP-array in a cohort of Armenian children with mental retardation

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The genetic diagnostics of mental retardation (MR) is difficult to establish and presently many cases remain undiagnosed and unexplained. Over the last few years, rearrangements below the detection level of conventional karyotyping have been proved to contribute significantly to the cause of MR. The implementation of SNP-array has enabled the high resolution analysis of copy number variants (CNVs) as duplications/deletions and uniparental disomy (UPD).
Here we report use of the SNP-array analysis for the first time in an Armenian cohort of 95 children with MR who had no visible cytogenetic abnormalities ascertained by standard karyotyping as well as microdeletion/microduplication, and subtelomeric abnormalities determined by FISH. We applied InfiniumHD whole-genome genotyping assay with the HumanCytoSNP-12 BeadChips (Illumina Inc.). Obtained data were analyzed with Illumina GenomeStudio and QuantiSNP software. Most of the detected CNVs have already been reported in the Database of Genomic Variants or recurrently present in our cohort. Recurrent microdeletion syndromes and UPD-associated syndromes were detected in nine cases (9.5%). Furthermore, 7 rare de novo CNVs (<5Mb) considered to be clinically relevant as well as 7 aberrations (>5Mb) also of potential clinical significance were discovered in syndromic and non-syndromic cases (16.8%) in chromosome regions, including 2p21-2p16.2, 6q22.1-6q22.32, 9q22.33-9q31.2, 10q26.11-10q26.3, 12q21.2-12q21.32, and 14q23.1-14q24.3. The majority of CNVs encompass several genes for which genotype-phenotype correlations have to be delineated.
Thus, in addition to its clinical diagnostic use in unexplained MRs, the introduction of SNP-array has facilitated identification of novel microdeletion/microduplication regions and corresponding disease genes.
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P03.028
Identification of two unrelated families with mentally retarded children extends the phenotypic spectrum of 15q25 deletions

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Microarrays can considerably contribute to the identification of novel microdeletion and microduplication syndromes. Deletions of 15q25.2-q25.3 have recently been associated with autism and schizophrenia in 3 patients. We report additional patients with these deletions from 2 unrelated families identified primarily due to mental retardation (MR). One patient is a 5 year old boy affected with high functioning autism, mild MR, obesity and speech delay. The second case is a 4 year old boy suffering from mild MR, microcephaly, speech delay and chorioretinitis. Karyotyping revealed a normal karyotype in both patients. SNP array analysis (Illumina HumanCytoSNP-12) demonstrated in both cases identical ~800 kb deletions of 15q25.2-q25.3 (chr15:82.7-83.5Mb (hg18)) flanked by low-copy repeats (LCRs). The deletions are adjacent to recurrent 15q25.2 deletions associated with congenital diaphragmatic hernia, cognitive deficits and Diamond-Blackfan anemia which are also flanked by LCRs. Chromosome 15q25.2-q25.3 has been predicted to be a hotspot for rearrangements based on its genomic architecture predisposing

to non-allelic homologous recombination. The region deleted in our patients encompasses 9 protein-coding genes, of which at least 3 are good candidates for the phenotypes observed. The ZNF592 gene product plays a role in the regulation of genes involved in brain development, the PDE8A gene is involved in learning and memory, and the NMB gene is associated with body weight regulation and obesity. The data currently available on the 15q25.2-q25.3 deletions suggest that they may represent another aberration from the growing list of genetic defects predisposing to neuropsychiatric phenotypes. Supported by CHERISH (EC FP7 223692) and MZOFNM2005.

P03.029
Molecular karyotyping of children with idiopathic mental retardation and/or dysmorphic features in Slovenian population

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Molecular karyotyping has become important tool for detecting chromosomal aberrations, which are overlooked with standard cytogenetic methods. Recently, it was proposed that arrayCGH should become first choice when unexplained developmental delay or congenital anomalies are involved.

Therefore, arrayCGH analysis of children with idiopathic mental retardation and/or dysmorphic features was performed to evaluate the importance of this diagnostic test in our laboratory. A series of karyotypically normal patients without subtelomeric abnormalities were included. In total, copy number variations (CNVs) in almost 45,5% of analysed patients were detected. CNVs with potential clinical significance were found in 18% of patients. Among them, 11% had aberration in regions with known microdeletion syndromes. In all cases, aberrations were causative for patients condition, therefore, they were determined to be pathogenic. Additionally, 7% of CNVs were characterized as probably pathogenic, based on their size and position. One male patient showed ~1,1 Mb duplication in region Xq21.32, and one female patient had ~2,3 Mb duplication in 8q23.1. Two additional patients had smaller deletions in 11q22.3 and 20q13.33 respectively. In 5,5% of patients, CNVs were identified as probably benign, based on their inheritance pattern. In the rest of the patients, aberrations were marked as variants of uncertain clinical significance. Further investigations should be carried out in these cases.

To conclude, diagnostic yield in our study was considerably higher (18%) than estimated average (~11%), due to the selected choice of patient. Nevertheless, we stress the importance of this test and emphasize the need of much bigger studies in Slovenian population.

P03.030
Epilepsy as a leading symptom in patients with mental retardation and chromosomal microdeletions/-duplications

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Epilepsy is defined as a chronic disorder of the brain, the occurrence of at least one epileptic seizure, and its neurobiological, cognitive, psychological, and social consequences, and is a common finding in chromosomal rearrangements detected by conventional karyotyping. Only few aberrations show a constant pattern of seizures. So, impairment of a single gene but also combined deleterious effects of several genes were assumed to be causative.

Here, we investigated 39 children with various patterns of epileptic seizures, mental retardation, and minor dysmorphisms and their healthy parents with the Illumina® Infinium Human1M-DuoV3 array to find *de novo* microrearrangements carrying epilepsy related genes. In four patients without any malformations or major dysmorphisms we found most likely causative *de novo* CNVs, i.e. deletions in 1q41q42.12 (3.3Mb), 19p13.2 (832kb), and Xq24 (165kb), and a two-part duplication in 17p13.2 (263kb) and 17p13.1 (464kb). One of the aberrations carries only one gene, which might be responsible for generalized tonic-clonic and myoclonic seizures and moderate developmental delay in the 4-year-old child. In four additional patients phenotypical consequences of the found *de novo* aberrations (deletions in 1q25.1 (71kb) and 19q13.43 (24kb) and duplications in 12q13.13 (116kb) and 15q25.1 (61kb)) cannot be excluded. Further clarification is in progress.

In total, the finding of causative chromosomal microrearrangements in a minimum of four out of 39 patients (10 %) with epilepsy and mental retardation but without major malformations shows again the power of DNA-arrays to find new disease-related genes and further expands the clinical indication for DNA-array investigations.

P03.031
A complex 21q deletion: molecular characterization marker chromosome by array-CGH.

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Introduction: Partial deletions of 21q are rare and these patients display a highly variable phenotype depending on size and position of the deletion. Proximal and distal deletions are associated with a milder phenotype, whereas deletions involving 31.2-36 Mb have a more severe phenotype.

Case report: We report a female four month old. During pregnancy a retarded growth intrauterine was detected. She was born in week 35 with a low birth weight for gestational age. At clinical examination mycrocephaly and dysmorphic features were noted including: mid-face hypoplasia, anteverted nares, retrognathia, hypertelorism, prominent eyes, broad base to nose and bulbous nasal tip and high palate.

The patient was hypertonic, ophthalmological and hearing examination showed strabismus and hypoacusia.

Magnetic resonance imaging revealed hypoplasia of corpus callosum and wide ventricles.

Results: Chromosome analysis showed a marker chromosome, the karyotype were reported as 46,XX,-21,+mar. A custom made array based on Agilent Technologies, called **KaryoArray®**, revealed two separate deleted segments: deletion 1 with a size of 21.65 Mb in 21q11.2-q22.12, and deletion 2 with a size of 4.04 in 21q22.2-q22.3. Extensive FISH analysis was performed on metaphase to validate the array-CGH results.

We review the literature and discussed about possible candidate genes.

P03.032
High frequency of genomic imbalances detected by array-CGH in patients with syndromic developmental eye anomalies

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Developmental eye anomalies (DEA) encompass a spectrum of severe structural defects of the eye caused by the disruption of the smooth process of ocular morphogenesis. With a birth prevalence of approximately 1 in 3,000-4,000, DEA are considered to account for at least 25% of childhood visual impairment worldwide. There are up to 400 different chromosome aberrations described associated to DEA in the various dysmorphology databases. The exact frequency

of chromosomal deletions and duplications causing DEA is unknown. With the advent of whole genome array-CGH analysis, the number especially of interstitial genomic imbalances increased dramatically.
Seventy patients with unexplained DEA associated to another congenital malformation or mental retardation were analyzed using oligonucleotide arrays (Agilent).
In 4 patients, clinically relevant deletions encompassing genes already known to be involved in eye development (FOXC1 and OTX2) were identified. In 4 other patients pathogenic deletions not classically associated to DEA were found: del(17)(p13.3p13.3), del(10)(p14p15.3) and del(16)(p11.2p11.2). The pathogenicity of 11 additional copy number variation (6 duplications and 5 deletions) is discussed. Altered segments ranged in size from 0.11 to 5.57 Mb. These results show that array-CGH provides a high diagnostic yield in patients with DEA syndromes and point to novel chromosomal regions associated with these conditions. Besides their importance for diagnosis and genetic counselling, these data may pave the way to the search of genes involved in eye development.

P03.033
Microdeletion of the Down syndrome critical region and duplication of the terminal section of chromosome 21 in a child with a distinct morphological phenotype

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We report on a child with a de novo combined interstitial deletion of 21q22.12-q22.3 and duplication of the terminal region of chromosome 21.
The patient is the 7th child of healthy parents. Birth weight and length were within a normal range, head circumference was below the 3rd percentile. Myocardial hypertrophy without clinical impact was detected, a finding that decreased over the years. At the age of 3 years and 10 months, microcephaly persists and weight and length are under the 3rd percentile. The boy presented with several dysmorphic features, e.g. brachycephaly with bitemporal narrowing, dysplastic, cup-shaped ears, hypertelorism, micropenis. There are feeding difficulties and regurgitations. He has a global developmental delay, especially speech development is impaired. There are an autistic-like manner and phases of behaviour problems with restlessness and laughter-attacks.
Molecular cytogenetic analysis of the subtelomeric regions identified a secondary signal of the chromosome 21 subtelomere probe on the distal short arm of one chromosome 21. An additional interstitial deletion was detected with a probe specific for the Down critical region and other region specific probes. The deletion encompasses 6.5 Mb distal to RUNX1 and the duplication 4.85 Mb as determined by tiling array-CGH analysis. STR marker analysis revealed that both the deletion and the insertion originate from the same parent. Both parents showed normal karyotypes.
Several of the clinical features reported in the patient can be assigned to the interstitial deletion of 21q whereas behavioural troubles described in the present case are not commonly mentioned.

P03.034
A first report of an unstably transmitted familial complex chromosome rearrangement

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Complex chromosome rearrangements (CCRs) are rare structural aberrations involving three or more breakpoints on two or more chromosomes. CCRs are classified as type I when they contain four or fewer breakpoints, or type II, when they contain more than four breakpoints. About one third of all CCRs are familial. Transmittance of such a CCR results either in non-disjunction at meiosis or is stably passed on to the next generation. Here we present a case of a phenotypically normal mother with a type II CCR involving chromosomes 1, 3 and 5 that gave birth to a phenotypically abnormal son. The boy presented with hypotonia and mild facial dysmorphisms. At the age of three he was severely mentally retarded, hypotonic and there were feeding disorders. Conventional karyotyping revealed the same CCR as previously was found in the mother. However, by use of array-CGH and FISH we discovered that during transmission of the CCR, a de novo deletion and duplication were evoked on two of the breakpoints by a mechanism of inversion/duplication on one of the derivative chromosomes. This is the first presented case of this mechanism in a structurally abnormal chromosome. Furthermore with this case we demonstrate the importance of breakpoint analysis in CCRs and stress that genetic counseling of a familial CCR is not straightforward.

P03.035
Clinical molecular karyotyping: an overview of 560 array experiments using 180k Agilent array.

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Array-based comparative genomic hybridization has become an indispensable tool in the detection of submicroscopic chromosomal aberrations in patients with multiple congenital anomalies and/or idiopathic mental retardation (MCA/MR). Once introduced into the diagnostics, it is gradually replacing karyotyping. It is now the first diagnostic tool of choice for patients with mental retardation and or multiple congenital abnormalities at the AMC in Amsterdam. Since the introduction of array CGH different resolutions have been used. For over a year now we have been using the 180k Agilent array in the diagnostics. An overview will be given. We have processed over 560 experiments consistent of both index (n=385) and family members (n=184) so far. Of the index patients almost 40% warranted additional testing of the parents or other family members to clarify the meaning of the aberrations that were found. We present a summery of our result to date, such as diagnostic yield, type of aberrations, size, number of aberrant probes and parental origin. An overview such as this is important as they may warrant change in the way the array data are analysed and interpreted.

P03.036
Interstitial deletion 13q: case report

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Chromosome 13q deletion is associated with varying phenotypes, which seem to depend on the location of the deleted segment. The most common clinical features are mental and growth retardation, malformations of brain, eye, kidney or heart, craniofacial dysmorphy and various congenital defects.
We report a 4 years old boy, patient of Neuropediatrics. Controlled pregnancy with threat of miscarriage in the second quarter. Birth weight: 3.100 g. He walked between 17 and 18 months and he didn´t crawl. He presents poor suck and swallowing reflex, bronchial hyperreactivity, hypotonia, hypertransaminemia, shy behaviour, and psychomotor and growth retardation. He has congenital absence of gallbladder and atopic dermatitis.
Conventional cytogenetic analysis using G-banding revealed a de *novo* 46,XY,del(13)(q?21q?31) karyotype. Whole Chromosome Paint (WCP) DNA FISH probe of chromosome 13 showed one pair of acrocentrics hybridized. 244K array-CGH characterization of chromosome 13q identified an 15.4 Mb deletion in 13q21.31-q22.3.
To date, there is still no consensus on possible correlations between the monosomy of distinct 13q regions and specific clinical features. Since 13q21 is a region with very few genes, deletion of band 13q22 is probably responsible of the patient´s phenotype.

P03.037
Genetic instabilities in the ataxia-telangiectasia brain: evidence for a neuroprotective effect of non-malignant genome/chromosome instability

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Ataxia-telangiectasia is a chromosome instability (CIN) syndrome caused by ATM mutations associated with DNA damage and repair defects. The role of CIN in this neurodegenerative disorder is incompletely understood. Molecular cytogenetic analysis allowed us identification of a dramatic increase in aneuploidy in the AT brain (Iourov er al., 2009). Additionally, degenerating cerebellum exhibited non-random breaks of interphase chromosomes and dramatically increased chromosome 7-, 14- and X-specific aneuploidy. CIN more severely affects the cerebellum than the cerebrum. Paradoxically, AT patients' longevity has positively correlated with CIN rates in the cerebellum. To solve this paradox we propose two alternative hypotheses: (1) overproduction of immature neural cells with unrealized chromosome breaks and aneuploidy takes place exclusively during early brain development, and chromosomally aberrant cells might be resistant to apoptotic clearance throughout ontogeny and, therefore, survive; (2) an age-dependent increase of the percentage of chromosomally defective cells could be explained by abnormal adult neurogenesis in the degenerating cerebellum during brain aging. Therefore, we have to admit that adult neurogenesis can occur in the diseased AT cerebellum. We suggest the longer the lifetime of AT individuals the bigger amount of immature neuron-like cells is accumulated in the degenerating cerebellum. The persistence and age-dependent accumulation of neural cells with chromosome breaks during ontogeny (even immature and chromosomally abnormal) could protect the diseased cerebellum against dramatic cell loss and, therefore, partially prevents the progression of neurodegeneration with possible neuroprotective effect. Finally, CIN represents an important genetic phenomenon mediating variety of cellular processes leading to neurodegeneration in brain.

P03.038
Identification of chromosome abnormalities in screening of a family with manic depression and psoriasis; predisposition to aneuploidy

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Manic depression (MD) is a severe psychiatric disorder that affects approximately %1 of the world's population. The etiology of MD is presently unknown, but epidemiological studies of families, twins and adoptions have consistently supported a genetic base. Some studies have supported the presence of autosomal dominant major loci. In this study, we aimed at detect the relationships between chromosomal aberrations (CAs) and immunological markers and MD. We used the cell cultivation and conventional G-banding. Random and non-random imbalances CAs were found in %51, %25 and %7-11 of cells. We found numerical abnormalities of chromosomes 8, 15, 21, 22, X and Y. However, structural aberrations consisted of duplications, deletions and translocations, with a focus on: loci on del(1)(q12-q23), del(1)(q21.1-q24), del(1)(q21.1-q23), del(10)(p11.2-pter), der(2) t(2;4)(p25;p12), t(2;22)(p14;p13), t(19;Y)? and dup(10)(q26). The susceptibility genes of MD may be located on these loci. Numerical sex CAs included 4(%4.6) with 45,X, 3(%4,3) with 47,XXY, and 4(%5,8) with structural chromosome X; del(X)(q13); del(X)(p11-pter) del(X)(q21.3) and inv(Y)(q11.2). The other characteristic finding was the presence of marker chromosome. The percentage of CD2+, CD4+ and CD8+ lymphocytes of the father were significantly higher, whereas CD4+ lymphocytes were decreased in the mather. The percentage of CD4 level of the son were decreased, whereas CD8+ lymphocytes were higher. These results may suggest that and MD have a significant impact on both genetic and immunological parameters, and continuing anti-inflammatory state in the family and this process may explain the cause of MD.

P03.039
Cytogenetic evaluation in congenital Blepharophimosis ptosis epicanthus inversus syndrome patients

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BPES is a rare eye genetic disorder which either occurs sporadically or familial. BPES is a genetic disorder which generally displays an autosomal dominant inheritance pattern. The purpose of this study is to identify chromosomal abnormalities in patients with congenital BPES. It has been shown that penetrance is 100% in type I when the transmission is by males only and 96.5% in type II in which transmission occurs through both sexes. In this study 20 sporadic and 3 familial cases of congenital BPES were enrolled for cytogenetic analysis. In all familial cases pedigree showed autosomal dominant pattern of inheritance. Disease information and all medical records were reviewed. Peripheral blood was collected and lymphocyte cultures were set up for chromosomal analysis. 20 metaphases were analyzed using GTG banding. Review of reported cases concluded that locus for eyelid development is situated at the interface of long arm of chromosome 3. Various reports linked the deletions (3q21, 3q22, 3q23, 3q24, 3q25) and translocations [t(3:7)(q26-qter:q+), t(X:3)(p22:q21), t(3:8)(q23:p22.1)] to the BPES. Thus cytogenetically different deletions and translocations of chromosome 3 have been described in patients with BPES. Deletions of 3q24-25 and 3q26.3 regions were found in two familial cases. We also found 3q-ter, 3q24-25 and 3q26-qter region deletions in three sporadic cases. 3qter region deletion found in 2 cases has been already been linked to the BPES. Theses finding may represent a severe manifestation of the disease. BPES is a heterogeneous entity, and evaluation and counseling of affected individuals should be undertaken with caution.

P03.040
Breakpoint characterization in a patient with a complex rearrangement of chromosome 7 including a dup(7)(q22.1q32.2) and an inv(7)(q31.31q31.33)

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For the understanding of the impact of small chromosomal rearrangements, the knowledge of the exact breakpoint-position is important, but breakpoint characterization on the base pair level is rare to find in the literature. However, by high resolution CGH- or SNP-arrays it is possible to localize the breakpoint region to a size that can be detected by simple PCR techniques. Here, we report on the breakpoint characterization of a *de novo* 46,XY,der(7)(pter->q31.33::q31.31->q22.1::q32.2->q31.33::q31.31->q32.2::q32.2->qter) karyotype in a 12-year-old boy with multiple congenital anomalies and mental retardation.

The size and the breakpoints of the duplication were narrowed with the Illumina® Infinium HD HumanOmni1-Quad v1.0 BeadChip, which covers more than 1 million SNPs, between rs17720576 (98.616.657 bp) and rs10275844 (98.621.315 bp) and rs10954272 (130.296.528 bp) and rs6467310 (130.313.744 bp), respectively. So, the size of the duplication as a whole is about 31,7 Mb. Breakpoint-spanning long-range PCR revealed a PCR product of approximately 18 kb. Sequencing of this product is in progress and should identify the duplication breakpoints on the base pair level.

The distal breakpoint of the inversion which is about 25 Mb in size was narrowed by fluorescence in situ hybridization (FISH) with the breakpoint-spanning probe RP11-111J7 (BlueGnome®, Cambridge, UK) between 117.631.652 bp and 117.790.729 bp. Fine-mapping of the proximal breakpoint of the inversion by FISH is in progress.

In conclusion, combination of FISH and high-resolution CGH- or SNP-arrays allows narrowing the breakpoints in complex chromosome rearrangements more easily than previously applied techniques. This could be important for a better genotype-phenotype correlation.

P03.041
Recurrence of cat-eye syndrome secondary to parental somatic mosaicism

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We report on the observation of 2 brothers presenting the association of an anal atresia, preauriculat tags and growth hormone deficiency. Their ophthalmologic examination was normal. Their father was asymptomatic. Their mother presented bilateral preauricular pit. But she had no history of anal atresia or short stature. On blood karyotype, the 2 boys had a supernumerary marker of the chromosome 22 on all the analysed cells. Their father had normal blood karyotype. Their mother presented only on few lymphocytes the same supernumerary marker.

This observation is original if we consider the recurrence secondary to parental somatic mosaicism. The growth hormone deficiency is also an unusual feature of this disease.

P03.042
Chromosomal imbalance detected by aCGH in a female with obesity and schizoaffective disturbance

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We present a 29 years old female with androgynous body evaluated for epimenorrhagia. At examination the patient showed minor anomalies in facial appearance: almond shaped eyed, abnormal aspect of the inner angle of the eyebrows, arched like upper lip. Her height was 168 cm, weight-117 kg, body mass index-41.45, head circumference-58 cm. Other findings were mental retardation, square hands with brachydactyly.

Developmental milestones were delayed, with sitting at 24 months and walking at 4 years, babbling at the age of 5 years. At the age of 18, the patient's behaviour showed cyclic maniacal changes, needing psychiatric assessment. Endocrinlogic markers for pituitary gland and cortisol were within the normal values.

Cytogenetic analysis showed a normal 46,XX karyotype. Due to morbid obesity, FISH analysis using Prader-Willi/Angelman syndrome probe was done, but the result was negative. Subsequently, because of the psychiatric disturbances we suspected a possible genomic imbalance and aCGH was performed (244k). Several duplications or deletions of very small size with poor gene regions were detected on chromosomes 11p, 10q, 19q and 21q. A small duplication containing several genes was detected on 22q13.33 region. For the genes in this region we did not find any correlations with the phenotype of our patient but rather would fit into manifestations for SHANK3 gene located in the same chromosomal region but further towards the telomere. Also a small duplication of 16-33 kb involving LHX4 gene was identified on chromosome 1q25.3, but clinical and the investigations do not sustain Machinis syndrome determined by LHX4 gene mutations.

P03.043
Chromosomal analysis in 1450 couples with reproductive disorders

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Most reproductive disorders have underlying chromosomal imbalance as a major cause for spontaneous pregnancy loss, infertility and childhood disability thereby, contributing significantly to the genetic burden on society. The diagnosis of chromosomal anomalies is made by karyotyping of GTG banded chromosome preparations obtained from whole blood. In the present study, conventional cytogenetic analysis was conducted in peripheral blood samples from 1450 couples with history of spontaneous abortions, mainly in the first trimester and infertility. Cytogenetic analysis revealed chromosomal abnormalities in 585 cases. Balanced reciprocal translocations were observed in 262 cases while Robertsonian translocations were observed in 11 cases. Apart from this, inversions in chromosomes 3, 17, X, Y and deletion of X chromosome was also observed. Novel cytogenetic anomalies like t(4;12), t(7;16) and t(1;9;10) were also observed that have not yet been reported in literature, to the best of our knowledge.. Some cases revealed heteromorphic variants in chromosome 1 (n=96) and 9 (n=102). The detection of these novel chromosomal anomalies reiterates the importance of conducting cytogenetic analysis in couples with history of reproductive failure.

P03.044
Gene Expression Profiling of Karyotypically Normal, Trisomy 12 and XXY Human Embryonic Stem Cells

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Human embryonic stem cells (hESCs) are well known to have the ability to proliferate unlimitedly and to differentiate into all cell types. Recently, a number of reports have been published in which chromosomal abnormalities has been observed in hESCs. We analyzed gene expression profiles of normal cell lines (SNUhES3: S3 and SNUhES4: S4), two variants of SNUhES3 (trisomy 12: S3v12 and XXY: S3vX), and one variant of SNUhES4 (trisomy 12: S4v12) using the Illumina HumanRef-6 BeadChip including 48,095 probe sets to find epigenetic difference between normal cells and chromosome variants. Gene expression patterns were showed that 55 genes were up-regulated and 28 genes were down-regulated in S3v12 to compare with S3 and 98 up-regulated genes and 75 down-regulated genes were identified in S3vX. Also, 87 genes were up-regulated and 61 genes were down-regulated in S4v12 to compare with S4. In particularly, three genes, PUS7L (synthetase), INHBE (growth factor) and PKIB (kinase inhibitor), were up-regulated and two genes, ERG1 (KRAB box transcription factor) and GALNT9 (glycosyltransferase) were down-regulated in S3v12, S4v12 and S3vX compared to normal hESC lines. Gene expression profiling in variant hESC lines with trisomy 12 or XXY chromosome as well as normal hESC lines may provide insights to the development of research field for cell differentiation and clinical approach such as cell replacement therapy.

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P03.045
Novel and known chromosomal imbalances associated with congenital heart defects found by screening with a SNP-array

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Cardiac development is controlled by complex genetic and/or environmental interactions. In an effort to identify new genetic factors associated with congenital heart defects (CHD) we selected a subset of 118 patients on which we typed 660.000 SNPs for intensity and genotype using the Illumina 660W beadchip. 38 were syndromic (had at least another major malformation and/or developmental delay) and 80 had an isolated CHD. Typed CHD included Tetralogy of Fallot (TOF n=27), Ventricular and auricular septal defects (ASD and VSD; n=17; n=27), transposition of great vessels (TGV; n=19) and other (n=21). Of all the found imbalances we only considered those larger than 75 kb, include at least a known or predicted gene, and that do not overlap any imbalance found in the Database of Genomic Variants (DGV). Previously, we had screened for the 1.5-3 Mb 22q11.2 deletion and for frequent aneuploidies (13, 18, 21, X and Y chromosomes), and excluded patients that were positive from this study. 24% of patients with an isolated CHD and 34 % of those syndromic, were found to carry a putatively causative imbalance (12 deletions and 22 duplications). Among previously known recurrent syndromes we identified 3 patients with an 11q24 deletion (Jacobsen syndrome); 3 patients with atypical 22q11.2 imbalances; 1 patient with 17q21.31 deletion syndrome and 1 with a 17q12 duplication. Some of the novel, previously not described imbalances were found to include suggestive candidate genes. Although further studies are needed, our study underpins the important role that submicroscopic deletions and duplications play in CHD.

P03.046
Telomere shortening in COPD: potential protective effects of coffee consumption

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Introduction: Chronic obstructive pulmonary disease (COPD) is characterized by incompletely reversible airflow obstruction, and chronic systemic oxidative stresss and inflammation play a major role in the pathophysiology of COPD. Telomere length has been observed to be inversely associated with increased oxidative stress and inflammation. Since chronic oxidative stress plays a major role in COPD, telomere length is hypothesized to reduce at a faster rate in COPD. The first aim of this study was to investigate telomere length in COPD patients in relation to oxidative stress and inflammation. The second aim of this study was to investigate the effects of caffeine, coffee, tea and alcohol intake on telomere length in COPD patients and healthy controls.

Methods: We measured telomere length in PBMC's of 89 COPD patients and compared these with 93 healthy, (ex-)smoking controls. In addition, the cytokines IL-8, IL-6 and TNF-α were determined, as well as CRP, SOD and plasma homocysteine.

Results and conclusions: We found that telomere length was shorter in COPD patients compared to controls. Cytokine plasma levels as well as CRP and homocysteine plamsa levels were inreased in COPD patients and SOD activity was decreased, indicating elevated oxidative stress in COPD patients. In addition, this study provides a first indication of the possible beneficial effects of coffee consumption and negative effects of alcohol consumption on telomere length. Future studies should focus on interventions with dietary antioxidants or anti-inflammatory compounds, such as those found in coffee, and their possible beneficial health effects in patients with chronic inflammatory diseases.

P03.047
Contiguous interstitial deletion encompassing both PTEN and BMPR1A associated with Cowden Syndrome

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We report on a patient with contiguous interstitial germline deletion of chromosome 10q23 encompassing BMPR1A and PTEN, with clinical manifestations of Juvenile polyposis and Cowden syndrome. The patient presented dysmorphic features including macrocephaly, wide fontanel with partly opened structures, wide forehead with prominent frontal protuberances, coarse features of the face with wide carp-shaped mouth, hypoplastic midface, small nose, retrognathia, prominent furrowed tongue, long philtrum, small low set ears, short neck, bilateral cryptorchidism, broad fingers with deep creases over small joints, short dorsally placed hallux and developmental delay at the age of 5 months. Multiple colonic polyps were diagnosed at the age of 3 years, following an episode of severe abdominal pain and intestinal bleeding. Comparative genomic hybridization revealed a deletion of 6.12MB in the chromosome arm 10q23. Genotyping using 5 microsatellite markers confirmed a *de novo* deletion on the allele originating from the father encompassing both PTEN and BMPR1A genes. The karyotype analysis additionally identified a balanced translocation involving chromosomes 5q and 7q, and inversion at chromosome 2: 46,XY,t(5q;7q)(q13.3-q36), inv(2)(p25q34). The mother was exposed to X-irradiation within the first 2 weeks of pregnancy which could explain the multiple genetic and phenotypic defects as well as the presence of chromosomal rearrangement in all analyzed cells. Current clinical findings and deletion of BMPR1A point to Juvenile Polyposis, but the dysmorphic features and the PTEN loss, strongly suggest a diagnosis of Cowden Syndrome which should be confirmed later in life with development of late onset phenotypic features.

P03.048
Cytogenetic investigation among patients with primary amenorrhea

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One of the sex chromosome aberrations in women is Turner's syndrome, which is much less common than other sex chromosome aneuploidies. The frequency of the Turner's syndrome is about 1 in 5000 live female births. The most frequent chromosome constitution in Turner's syndrome is 45,X. However, about 50 percent of cases have other karyorypes. About 25 percent of cases reveal mosaic karyotypes, in which only a proportion of cells show 45.X. The chromosome constitution is clinically significant, and different cases show rather different manifestations according to their karyotypes.

The typical abnormalities in Turner's syndrome include short stature, webbing of the neck, characteristic unusual face, a broad chest with widely spaced nipples, gonadal dysgenesis, low posterior hairline, and sometimes with renal and cadiovascular malformations or hearing loss.

A total of 267 cases were referred to our Department with primary amenorrhea. Cytogenetic studies were performed on peripheral blood cultured for 72 hours in the presence of PHA. For routine chromosome analysis, 20-30 Giemsa-banded cells were analyzed , with two cells karyotyped.

219 (82%) patients showed normal karyotypes. 48 (18%) patients showed X chromosome abnormalities.

21 (43.7%) cases revealed 45,X. 11 (23%) cases were either 45,X/46,XX or 45,X/46,XY mosaics.

9 (18.7%) cases showed either 46,X,i(Xq) or 45,X/46,X,i(Xq) mosaics.

The rest of 7 (14.6%)cases include: 46,X,t(X;19)(q22;q13) [two cases]; 46,X,delX(q13) [two cases]; 47,X,-X+derXp,derXq; 47,XXX; and 45,X/47,XXX mosaics.

P03.049
A familial translocation t(14;21) in four generations affected by Trisomy 21

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INTRODUCTION: Only 4% of Down Syndrome (DS) occurs whit a homologous or heterologous translocation (13q, 14q, 15q or 22q) of chromosome 21, of which 1% of all DS are familiar. This type of aneuploidies is caused by the incorrect distribution of homologous chromosomes during meiosis.

PATIENTS AND METHODS: In 149 from 600 pregnancies between 2007-2009 was requested molecular testing for early aneuploidy screening. 6 postnatal and 6 prenatal cytogenetic studies were carried out in individuals of three generations belonging to a family carrying a heterologous translocation. The DNA obtained from 3 related prenatal and 2 postnatal samples were amplified for 7 hypervariable genetic markers in chromosome 21 using QF-PCR techniques.

RESULTS: We identified one family carrying the t(14;21) translocation with 5 affected by DS, 9 non-affected carriers and 5 abortions. The definition of associated markers to translocated chromosome 21 in an affected individual, revealed identical genetic makeup except for the more distally located. The third chromosome 21 (paternal) differed in all markers with the two previous.

The pedigree identified a set of individuals at possible risk of fathering children with DS.

CONCLUSIONS: The origin of trisomy 21 was maternal, but double-recombination in meiosis I generated an exchange of genetic material translocated, except the most telomeric region, before the incorrect distribution of homologous chromosomes 21 in meiosis II.

Our results identified an unusual mechanism of double-recombination of the extra chromosome and expose the diversity of causal etiology.

P03.050
Mosaic interstitial duplication 17q

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Interstitial duplications in the long arm of chromosome 17 are rarely encountered. We report a female patient who was born with multiple congenital anomalies. She had loose skin in the neck, narrow forehead, depressed nasal bridge, long philtrum, split uvula, a sacral dimple, rhizomelic shortening of all extremities, postaxial polydactyly of hands and feet, bilateral pes equinovarus and extreme hypermobility of the large joints.

Conventional karyotyping demonstrated a mosaic (87%) interstitial duplication in the long arm of chromosome 17 between q21 and q25. The duplication was also present in fibroblasts from skin (83%) and cartilage (97%). SNP array analysis was performed to characterize the duplication in more detail. Additional FISH analysis revealed that the duplication was direct. Both parents had a normal karyotype. Detailed molecular characterizations and reports of chromosomal imbalances in combination with clinical phenotypes are important for accurate genotype-phenotype correlations in genetic counseling.

P03.051
The Dyke Davidoff Masson Syndrome as the indicative feature in a child with a 22q11.2 deletion

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Cerebral hemiatrophy with homolateral hypertrophy of the skull and sinuses (resulting in facial asymmetry) and elevation of the sphenoid wing and petrous ridge, associated with contralateral hemiplegia, seizures, mental retardation, difficulty and impairment of speech development are features of the radiological syndrome: Dyke Davidoff Masson Syndrome (DDMS).

We report here, the case of 4 years old boy, with facial asymmetry, hemiparesis, mental retardation and epilepsy. The cerebral MRI showed a cerebral hemiatrophy and a fronto-parietal polymicrogyria (PMG). A cytogenetic study was performed on R-banded chromosomes showed a 46,XY formula. A fluorescence in-situ hybridizing (FISH) using specific *Tuple1* probe showed a 22q11.2 deletion, well characterized by array comparative genomic hybridization (CGH array) revealing a 3Mb 22q deletion, extended from 16,597 to 19,712Mb including *TBX1* gene.

The 22q11.2 deletion syndrome or velocardiofacial (VCF) syndrome is a microdeletion syndrome associating conotruncalcardiac malformation, palatal dysfunction or cleft, facial dysmorphism, immuno-deficiency, developmental delay and vascular anomalies. Several brain malformations as PMG have been described in association with VCF. These anomalies seem to result from abnormal cortical organisation caused by ischemia occurring during a critical period of cortical development. The *TBX1* gene on 22q11.2 encodes T-box1 protein, necessary for the normal development of large arteries, could be a good candidate for DDMS. The deletion of this gene in our case could then explain the PMG observed in the frontal and parietal lobes. This data, improves also the check of the 22q11.2 deletion in DDMS or PMG cases even in the absence of other VCF syndrome features.

P03.052
Rapid Prenatal Diagnosis of Trisomy 18 and 21 by Fluorescence in Situ Hybridization (FISH) on Uncultured Amniotic Fluid Cells

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Fluorescence in situ hybridization (FISH) in uncultured amniotic fluid samples is a useful tool for rapid prenatal diagnosis in pregnancy with high risk for chromosomal abnormality. FISH using specific probes for chromosome 18 and 21 was performed in 175 amniotic fluid samples referred to the Institute of Reproductive Medicine and Population, Seoul National University. All of the FISH results were compared with the karyotypes from conventional cytogenetic analysis. Amniotic fluid samples were obtained between the 16th and 28th weeks of gestation. The most frequent indication performed FISH analysis was the ultrasonographic (US) findings of fetal anomalies (54.6%). Aneuploidy was identified in 6 out of 175 uncultured amniotic fluid samples by FISH. Three cases of trisomy 18 and three cases of trisomy 21 were detected. 4 out of 6 cases with aneuploidy were with structural abnormalities at US. Conventional cytogenetic results revealed 4 chromosomal abnormalities not detectable by FISH such as 47,XXY, 45,X/46,XX mosaicism, balanced reciprocal translocation, and marker chromosome. These results suggest that FISH in uncultured amniotic fluid samples indicated the abnormal US findings in fetus is a reliable method for the rapid prenatal diagnosis of trisomy 18 and trisomy 21. FISH analysis should generally be used as an adjunct to the conventional cytogenetic analysis and not be used alone. Genetic counseling for prenatal diagnosis is important for patient's understanding about the limitations of FISH for detection of aneuploidy in uncultured amniotic fluid cells.

P03.053
‚Inherited complex mosaicism associated with fragile site at 16q22'

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The fragile site of 16q22 is found in 1%-5% of individuals. We report a case of familial chromosomal mosaic fragile site 16q22 in a 17-year-old caucasian adolescent girl with secondary amenorrhea. Pedigree data showed a few cases with menstrual disorders from father's side. Hirsuitism in spine, upper and lower limbs and linea alba region was observed during physical examination of proband. Pelvic and thyroid ultrasonography did not show any abnormalities. Serum hormones levels were in reference ranges.

Conventional G-banded karyotype analysis from GTG banded metaphase chromosomes revealed chromosomal rearrangements in the girl and her father such as: mos 46,XX,del(16)(q22)[8]/ 46,XX,fra(16)(q22)[11]/ 46,XX[31] and mos 46,XY,del(16)(q22)[5]/ 47,XY,del(16)(q22),+ace(16)(q22qter)[3]/ 46,XY,fra(16)(q22)[12]/46,XY[30], respectively. Chromosomal analyses of the proband's mother showed normal karyotype. We used subtelomeric FISH for the chromosome 16 to confirm and determine ratio of these cell clones. FISH analysis showed no del(16)(q22) since we observed both signals of chromosome 16q. One signal of a long arm was detected on the acentric fragment of chromosome 16. FISH analysis of patient's parents indicated fragile chromosome 16 was inherited from patient's father. QT-PCR testing for sex chromosomes aneuploidies was performed. Normal result was obtained, sex chromosomes XX.

P03.054
Whole-genome array-CGH screening in autosomal dominant sensorineural hearing loss patients detects two chromosomal alterations at 5q32 and 7q31.1

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Hereditary hearing loss is a clinically and genetically heterogeneous sensory disorder. In 20% of cases is inherited in an autosomal dominant pattern with no additional clinical signs (ADNSHL). To date, 59 loci for ADNSHL have been mapped to chromosomal regions. Due to tremendous genetic heterogeneity, the identification of genes that affect the process of hearing has been challenging, so in order to verify whether genomic alterations contributed to the hearing loss etiology and to discover novel DFNA locus, we investigated 40 patients with ADNSHL by oligonucleotide array-CGH. Copy number alterations were detected in two ADNSHL patients (5%), both alterations inherited from their affected fathers. The first one, a 56 Kb deletion on 5q32 region, encompasses only one gene, the POU4F3. Mutations in this gene had already been associated with hearing loss in other four cases; the total deletion of the POU4F3 had not been reported. POU4F3 protein has an important role in the maturation, differentiation and survival of cochlear hair cells, defects in these cells may therefore

explain sensorineural hearing loss. The second alteration was a 438 Kb duplication located on 7q31.1 region affecting two genes: IMMP2L and DOCK4. In this case the DOCK4 is a strong candidate gene for causing the ADNSHL since previous studies indicate that a DOCK4 harmonin-activated signaling pathway regulates actin cytoskeleton organization in stereocilia. We report for the first time a case of ADNSHL caused by deletion of the entire POU4F3 gene, and point DOCK4 as a deafness gene in representing a novel DFNA locus.

P03.055
Possible association of heterochromatin variants in human karyotype with reproduction failure

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Heterochromatin abnormalities of chromosomes are believed to be clinically insignificant variants of the human karyotype. Chromosomes with areas of constitutive heterochromatin (chromosomes 1, 9, 16 and Y) are commonly involved. However, several authors have studied the possible association of heterochromatin variants with selected clinical diagnoses, especially with reproduction failure (sterility and/or repeated abortions).

We have compared the incidence of heterochromatin variants in the group of patients with reproduction failure to the incidence in the control group. The study group was composed of 761 patients (93 individuals and 334 couples) with reproduction failure diagnosis karyotyped between 2007 and 2009 in our cytogenetic laboratory. The control group was composed of 885 fetuses who were karyotyped only because of the advanced age (35 years or more) of their mothers between 2003 and 2009 in our laboratory; cases with unequivocally pathologic findings were not included.

Heterochromatin variants have been found in 106 patients (13.9%) in the study group and only in 74 individuals (8.7%) among controls. The difference was statistically significant ($p < 0.001$). The most common variants were those of chromosome 9 (including pericentric inversions); they were detected in 51 (6.7%) patients with reproduction failure but only in 32 (3.7%) fetuses. This difference was statically significant ($p < 0.01$), too. There was no significant difference in the incidence of heterochromatin variants in men and women karyotyped for reproduction failure.

Our results in Czech population correspond to findings of other authors. However, the causal mechanism for these observations remains unknown for now.

P03.056
Karyotype instability in morphologically abnormal culture of mesenchymal stem cells and PHA-stimulated lymphocytes of the patient

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The results of cytogenetic study of one bone-marrow derived mesenchymal stem cell (MSC) culture, distinguished from other 200 cultures from donors in stem cell bank by its abnormal morphology are reported. This culture demonstrated spontaneous disturbances in morphology and proliferation at the early passages - cells grew in size, lost adhesion properties, detached from the flask surface, and persistently divided in the suspension. They also showed up-regulated expression of CD106. The donor of this culture had no clinical or laboratory features of bone marrow pathology

We analyzed 58 metaphases of the abnormal MSC culture and revealed various genomic and chromosomal mutations, including tetraploidy, additional marker chromosomes, monosomy 2,8,13,14,15,16,17,21, insertions of chromosome7; derivatives of chromosomes 3,7,9,17, deletions of chromosomes 2,14. Cells with abnormal karyotypes accounted for 22% of all cells studied. No repeatability of karyotype aberrations was observed, which could be attributed to their single cell origin.

The cytogenetic analysis of PHA-stimulated lymphocytes of the same patient showed that 14,4% of cells (23 from 160) featured abnormal karyotype. The cells with polyploidy, tetrasomy 21, double trisomy(+10,+20), trisomy21, double monosomy(-2,-10,), monosomy 18,21,22,Y; derivatives of chromosomes 4,14, isochromosome 9, translocation between chromosomes 8 and 10, homogeneously stained regions in chromosomes 1and4 were registered. Monosomy Y was detected in 5cells and trisomy 21 in 2 cells. Thus, concordance in karyotype aberrations in MSC and lymphocytes was showed for chromosomes 2,14and21.

In conclusion, karyotype aberrations, revealed both in MSC and lymphocytes substantiate genome instability in this particular patient.

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P03.057
Co-localization of acetylated histone H3 and methylated DNA demonstrates band and stage-specificity in distribution in human metaphase chromosomes

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Distribution of acetylated histone H3 (AcH3K9) in human metaphase chromosomes from lymphocytes of adults was studied. 60 DAPI-stained metaphases (~400 bands per haploid genome) from 5 individuals were analyzed with antibodies against AcH3K9 (Abcam, USA). AcH3K9-rich regions (the brightest AcH3K9-fluorescence) corresponded to 32 R-bands. Medium to weak fluorescent AcH3K9-signals were detected in 86 R-bands. All G-bands demonstrated weak AcH3K9-fluorescence. AcH3K9 was absent in heterochromatic blocks of chromosomes 1, 9, 16, Y.

This H3K9 acetylation profile was compared to the relevant DNA methylation patterns after 5-methylcytosine antibodies staining. Non-acetylated heterochromatic regions were hypermethylated. Among R-bands with medium to weak acetylation 6 demonstrated weak, 48 - medium and 32 - high DNA methylation level. 32 AcH3K9-rich R-bands showed variable DNA methylation levels: 17 - high, 12 - medium and 3 (1p13, 2q31, 2q33) - low. Low level of DNA methylation in highly acetylated bands favors their specific function in lymphocytes.

Both H3K9 acetylation and DNA methylation were also studied in lymphocyte chromosomes from fetal cord blood. Two highly methylated regions 2q31 and 2q33 and one hypoacetylated one - 2q31 - distinguished fetal chromosomes from their counterparts in adult.

The co-location of acetylated and methylated chromosome patterns suggests feasible distribution of active and inactivated chromatin regions which looks to be band and stage specific.

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P03.058
Case report: A male with two idic(Y) chromosomes

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Idic(Y) cause several sex-linked phenotypes ranging from typical Turner syndrome, to phenotypic males, and to those with ambiguous genitalia. We report a 17-year-old boy with male development, hypogenitalism, small stature, slight obesity and normal inteligence. Cytogenetic analysis and GTG-banding were performed on metaphases from cultured peripheral blood lymphocytes. As a result, the lymphocyte culture revealed a karyotype 45,X with a pericentric inversion of chromosome 9 of maternal origin. Chromosome analysis was also performed on fibroblasts from the skin. Two cell lines were observed: 45,X,inv(9)mat[18] and 47,X, inv(9)mat,+marx2[17] Fluorescent in situ hybridization (FISH) analysis was performed on fibroblasts using the probe for the chromosome X centromere (DXZ1) and the DYZ3 probe for chromosome Y centromere according to the manufacturer's instructions. The presence of four Y-centromeric signals and an X-centromeric signal was observed in 40% of cells while in the remaining 60% of the metaphase cells only one signal for the X chromosome

centromere was present. Dual color FISH with an SRY (Yp11.3) DYZ3 (YCEN) and DYZ1 (Yq12) probes demonstrated the presence of two centromeres and two SRY regions per one marker. The probe for the Y heterochromatic region DYZ1 didn't show any signal. Thus, the patient karyotype was mos 45,X,inv(9)mat[18]/47,X,+idic(Y)(q11) x2dn. ish idic(Y)(q11)(SRY++,DYZ3++,DYZ1-)x2[10]. Only several patients with a cell line containing two isodicentric Y-chromosomes with a break in Yq resulting in tetrasomy of Yp have been described. Correlation between phenotype and karyotype is not yet well defined, this clinical report will be helpful in defining the phenotypic range of this chromosomal abnormality.

P03.059
Tetrasomy 15q in a girl with epilepsy and hypomelanosis

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The inv dup (15) or idic (15) is the most common of the heterogeneous group of the extra structurally abnormal chromosomes (ESAC). Two cytogenetics types have been identified. One is a metacentric or submetacentric and heterochromatic chromosome, not containing the Prader-Willi Syndrome/ Angelman Syndrome Critical Region (PWS/ASCR) [dic(15)(q11).dic(15)(q11)], can be familial or *de novo* and most children show a normal phenotype. The second type has 15q euchromatin including PWS/ASCR [dic(15)(q12 or q13)], that frequently derives from the two homologous maternal chromosomes at meiosis. This condition is associated with an abnormal phenotype, which constitutes the idic(15) syndrome, and is nearly always sporadic. The inv dup (15) displays distinctive clinical findings represented by early central hypotonia and intellectual disability, epilepsy and autistic behaviour. Expressive language is absent or very poor and often echolalic. Epilepsy with a wide variety of seizures, as a typical Lennox-Gastaut Syndrome (LGS), can occur in these individuals. Areas of increased and reduced skin pigmentation can be occasionally observed.

We report on a 6 years old girl, patient of Neuropediatrics, with language delay, LGS and hypopigmented streaks on the trunk. Cytogenetic studies and FISH analysis showed that she had a de novo 46,XX,+idic(15)(q13) karyotype. Cytogenetic analysis confirm the diagnosis of idic(15) syndrome in our patient.

P03.060
A *de novo* 9Mb deletion into an inherited 2q paracentric inversion gives rise to severe developmental delay and eyelids malformation

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Our patient, a girl, presented at 14 months- with developmental delay and hypotonia. Her clinical examination also displayed ptosis, blepharophimosis, and a toe malformation. Her karyotype indicated, an apparently balanced 2q paracentric inversion (46, XX, inv(2)(q21q37)) inherited from her normal father, and confirmed by FISH. In addition, Agilent 105k array-CGH proved positive for a 9Mb deletion (2q31.1q31.3) within the inverted region in the index patient. By contrast, array-CGH was normal in her normal father also harboring the 2q31 paracentric inversion. Its *de novo* character was confirmed by FISH and qPCR.

An interstitial (an not terminal) secondary rearrangement occurring within an inverted segment is a rare event that is not readily explained by non allelic homologous recombination (NAHR). The advent of array-CGH as an additional mandatory investigation in case of abnormal phenotype and an apparently balanced translocation has shown in a number of cases an additional loss of chromosomal material in the close vicinity of the breakpoints (neighboring regions). However, in the present case, the deletion occurred at distance of the breakpoints, suggesting an unusual mechanism. The phenotype of our patient deserves consideration, since an eyelid malformation has been also reported in the three patients with similar microdeletions retrieved from literature. More than 50 genes are included in the deleted interval and one can speculate on the involvement of one of them in eyelid differentiation. Our report confirms that developmental delay and malformed eyelids is suggestive of a 2q31 microdeletion syndrome, in addition to the well-known 3q23 BPES microdeletion syndrome.

P03.061
Familial segregation of an interchromosomal insertion (9;15)(q33;q21.1-q22.31) with three individuals carrying an unbalanced karyotype characterized by high resolution array

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Chromosome insertions are complex rearrangements occurring in about 1/5.000 live births. Originally balanced chromosome insertions, after meiotic segregation, can cause unbalanced structural rearrangements with deletions or duplications of the translocated segment, which explains the high risk (50%) of producing an abnormal offspring. Here, we report an interchromosomal insertion in three generations of a family. The proband presented neurological disorders, mitral stenosis, velopharyngeal insufficiency and facial dysmorphisms. The G-banded karyotype showed an insertion in the long arm of chromosome 9 in q33 region. His mother is carried of a balanced insertion [ins(9;15)(q33;q21.1-q22.31)]. The cytogenetic analysis of other family members identified six individuals carrying an apparently balanced rearrangement and two other members carrying the same unbalanced rearrangement [der(9)ins(9;15)(q33;q21.1-q22.31)]. Despite the clinical heterogeneity these individuals present some similar characteristics such as RDNPM, short stature and dysmorphic features. The high resolution array analyses (Genome-Wide Human Array 6.0,Affymetrix®) of three individuals with unbalanced karyotype revealed a duplication of 19.3Mb on chromosome 15 ranging from q21.1 to q22.31 [44275184-63567267 - hg18]. The array analyses of an apparently balanced carrier presenting mental retardation and speech delay showed normal results and is still in investigation. Despite some rare reports of overlapping duplications in 15q21.2-q22.31, this family herein described seems to be the first one with duplication of this specific region. The combined approach of karyotype and array analysis allowed the correct characterization of the rearrangement in most of the family members. **Financial support:** Fapesp and CNPq

P03.062
Prominent transformation of karyotype of human mesenchymal stem cells 4BL6 in long-term culture

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4BL6 cells were obtained from donors peripheral blood and were grown in standard monolayer culture. During the observation period (219 passages) the cells maintained fibroblast-like morphology, sensitivity to colchicine and proliferative potential. At 205-th passage 4BL6 cell population contained 36% of polyploid cells and 62.5% of neardiploid cells. Among polyploid cells, 81% were tetraploid and 12.5% were triploid. The number of chromosomes in neardiploid cells varied from 30 to 49. 80.8% of these cells contained 39-44 and 60% - 41-43 chromosomes (possible modal clone). The number of chromosomes in diploid and polyploid cells (42-43 and 84-85 respectively) and their ratio in culture (1.7 : 1) allows to assume that upon reaching 205-th passage, the reproduction of 4BL6 cell line occurs by symmetrical division of polyploid cells. To define the nature of chromosomal anomalies, GTG, QFH/AcD, Cytocell OctoChrom stainings and array CGH were used. X monosomy, t(1;11)(q12;p15) and t(2;?)(q11;?) were defined as most common aberrations and observed in 80%, 65% and 46% of metaphases respectively. 23% of cells contained t(5;15)(p10;q10) and 10% - t(5;15)(p10;q10), that were never registered together. Chromosomes 3, 6, 7, 8, 9, 14, 18, 20, 21 and 22 showed normal number and constitution, whereas chromosomes 1, 2, 4, 10, 12, 13, 16, 17, 19 demonstrated unbalanced structural rearrangements (deletions/duplications) from 0.6 to 10 and more Mb. In our opinion, long-term transformation of the 4BL6 cell line

occured due to formation of typical chromosomal rearrangements that changed the dose of genes-suppressors of cancer growth and genes-inducers of proliferation.

P03.063
Congenital stationary night blindness, high myopia and severe mental retardation: search for a homozygous microdeletion 15q13.3 !

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Heterozygous microdeletions 15q13.3 are not rare and have been associated, among other findings, with intellectual disability, autism, and schizophrenia. It has also been found in controls and healthy relatives of affected individuals. The reason for this wide phenotypical variability is poorly understood. On the contrary, homozygous deletions of this same area have so far been reported in only 4 patients and seem to be linked with a specific clinical presentation, namely severe mental retardation, seizures and visual impairment.

The 12-year-old female patient was born at term with normal growth parameters. Absent visual pursuit was noticed at 2 months and congenital stationary night blindness (CSNB) was diagnosed at 9 months by electroretinography. At 10 months, severe developmental delay was evident and seizures were noticed. High myopia (-10 dioptries) is known since the age of 21 months. Karyotype, methylation of the AS/PWS region and MECP2 analysis were normal. Array CGH (Agilent 244K) revealed a homozygous 1.5 Mb 15q13.3 deletion (28719136-3029809 bp, UCSC Hg19). The deleted interval comprises 6 genes: MTMR15, MTMR10, KLF13, OTUD7A, CHRNA7, TRPM1. A homozygous mutation of TRPM1 has been recently associated with CSNB and myopia (Audo et al., AJHG 2009; 85: 720-729). The asymptomatic parents are both heterozygous carriers.

This report confirms that the clinical picture of homozygous 15q13.3 deletions is very homogeneous and comprises, besides severe mental retardation and seizures, CSNB with myopia as a key feature. It also proves that CSNB due to TRPM1 recessive mutations is caused by a loss of function of the coded protein.

P03.064
Delineation of the Interstitial 6q25 Microdeletion Syndrome: refinement of the critical causative region

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Cryptic chromosomal aberrations are associated with various syndromes. Microdeletion of the long arm of chromosome 6q is rare but specific clinical entity. Phenotypic presentation includes mental retardation, acquired microcephaly, dysmorphic features, structural brain malformations and other features. The smallest reported region of microdeletion responsible for the phenotype was mapped between 6q24 and 6q27 and spans >3MB with more than 12 coding genes.

A 2 years old boy presented with mental retardation, acquired microcephaly, dysmorphic features and dysgenesis of corpus callosum. He has deep set eyes, prominent forehead, posteriorly rotated ears, short neck, short proximal phalanges, transverse creases, fetal pads and hypoplastic scrotum. Neurological examination revealed mixed axial hypotonia with limbs hypertonia.

He is the fourth child of an unrelated generally healthy couple. The mother is carrier of a Robertsonian translocation (14;15).

Our patient was found to be a carrier of the same translocation and his karyotype is 45 XY,der(14;15)(q10;q10)

Affymetrix Wnole-Genome 2.7M Array Chip revealed deletion of 1191 kbp at 6q25.3. The deleted region contains 2 coding genes:
ARID1B and ZDHHC14.

These genes are highly expressed and evolutionarily conserved. ARID1B encodes a subunit of chromatin-remodeling complex. The encoded protein is expressed in different brain regions during fetal development suggesting its important role in CNS development. ZDHHC14 belongs to a palmitoylating enzyme family proteins. Protein palmitoylation is crucial in CNS development and plays an important role in neurite outgrowth and synaptogenesis.

We found two attractive genes, whose haploinsufficiency caused the distinct phenotype of 6q deletion syndrome with dysgenesis of corpus callosum

P03.065
Detection efficiency of FISH vs MLPA method in microdeletion syndromes patients

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Microdeletion syndromes are complex genetic disorders caused by different changes of contiguous genes located in particular chromosomal regions. These changes are usually cryptic chromosomal aberrations - microdeletions or microduplications. The OMIM database currently contains more than 100 microdeletion/duplication syndromes of various types but due to the extensive phenotypic variation and overlapping phenotypes the correct diagnosis is often difficult to be determined.

We have analysed 64 probands with normal karyotype and specific clinical findings such as mental retardation or developmental delay combined with neonatal hypotonia, congenital anomalies or facial dysmorphism. We used the MLPA method to scan for cryptic rearrangements. Each proband was analysed by the P245 kit to detect 21 most common microdeletion syndromes. Additional microdeletion syndromes were analysed using the P297kit. Moreover, subtelomeric regions were analysed with the P036kit. Overall 10 positives (16%) were detected, 3 duplications and 7 deletions, respectively. The MLPA kit P245 was able to detect 9 of all found rearrangements. One abnormality was detected only by the MLPA kit P036.

We have used the FISH method with locus specific probes to confirm the findings and to compare the efficiency of both methods in cases of MLPA positive probands. Surprisingly, we have found the discrepancy in 2 cases. The deletion of 10p14-15 and 1p36 was detected by MLPA and not confirmed by FISH.

Although the application of FISH method is the matter of choice for the diagnostics of microdeletions, the MLPA method appears as more sensitive and more effective.

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P03.066
Contribution of cell cycle control genes epimutations to the origin of chromosomal mosaicism in human miscarriages: a model and experimental evidences

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Chromosomal mosaicism is a hallmark of human miscarriages. Among different factors underlying abnormal chromosome segregation the epimutations of cell cycle genes is of particular interest. Epigenetic reprogramming during early ontogeny may predispose to methylation abnormalities. We aimed to determine the mechanisms and stages of cytogenetic and epigenetic errors origin and suggest a model for identification of epimutations impact on mosaicism formation. Extraembryonic tissues of 69 diploid-aneuploid miscarriages, 20 diploid miscarriages and 22 induced abortions of similar gestational age were examined for epimutations of cell cycle control genes *RB1* and *P14ARF* which were hypermethylated in 42% and 10% of mosaic embryos, respectively. Cytotrophoblast and extraembryonic mesoderm appears a convenient model to determine ontogenetic stages of aneuploidy and epimutations origin because they are derived from different germ layers, isolate after implantation and differ in DNA methylation. Diploid-aneuploid mosaicism originates postzygotically due to trisomy rescue or de novo mitotic error. If mitotic errors occur before germ layers divergence then both tissues will be mosaic. Mosaicism will be confined by one tissue if mitotic mutation occurs after divergence. Epimutations may originate from demethylation and de novo methylation errors during epigenetic reprogramming. Analysis of all possible combinations of cytogenetic and epigenetic abnormalities underlies our model. Interpretation of the methylation role in the origin of mosaicism using the model allowed concluding that *RB1* and *P14ARF* epimutations are primary to diploid-aneuploid mosaicism in

at least 4,7% of mosaic embryos. This study was supported by Federal Program N P806, P1161 and State Contract for National Educational Center 02.740.11.0281.

P03.067
Use of MLPA as a screening method for patients with unidentified chromosomal abnormalities

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Multiplex ligation-dependent probe amplification (MLPA) is a variant of polymerase chain reaction that permits multiple targets to be amplified using a single primerpair. It is a robust, facile, flexible and inexpensive technique that can detect both deletions and duplications for more than 40 loci in one assay. Clinical applications of this modern method are extending quickly.

Due to the advantages mentioned above, we have introduced subtelomeric MLPA as a screening method (before more complex and expensive molecular methods) for patients with complex/ unidentified chromosomal rearrangements. Its' application will be illustrated for 6 cases with mental retardation and multiple congenital anomalies. The karyotype of the patients (G-banding) was abnormal, each one presenting an additional terminal chromosomal fragment (involving chromosome 4,9,15, 8).). Using MLPA we identified in 5 cases terminal duplications and in one case a known polymorphism (chromosome 15). Array comparative genomic hybridization (aCGH) was used in 3 cases to validate our MLPA results. All the abnormalities identified with subtelomeric MLPA have been confirmed, but in one case an extra defecthas been identified. Clinical features, karyotype, MLPA and aCGH results will be will be presented in detail.

In conclusion, we preset 6 cases with mental retardation associated with multiple congenital anomalies and abnormal karyotype in which subtelomeric MLPA contributed to the precise description of the chromosome abnormalities. Confirmation by array CGH provides evidence that MLPA is a very useful tool for the characterization of unbalanced karyotypes.

P03.068
Clinical and Molecular cytogenetic characterization of two cases of mosaic ring chromosome 13

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The mosaic ring chromosome 13 syndrome is a rare chromosomal abnormality. The mechanism of ring chromosome formation is usually associated with loss of genetic material.

We report 2 cases of mosaic ring chromosome 13. For the first patient, a male with a karyotype: 46, XY,r(13)[40]/45,XY, -13 [10]. He presented a delayed psychomotor development, mental retardation, dysmorphic features and bleeding disorders. The second is a case of prenatal diagnosis; completed with the postmortem autopsy of a foetus of 33 week of gestations. The genetic amniocentesis analysis showed a mosaic karyotype : 46, XY, r (13) [8]/45,XY,-13[5] ; which was different of that of the cordocentesis analysis: 46,XY, r(13)(q32q34)[18]/46,XY,dic r(13) (q32q34)[2]. Sonography showed intrauterine growth retardation, holoprosencephaly that have been confirmed by the autopsy report; which revealed additional anomalies such as the partial agenesis of the pancreas. The chromosomal terminal regions exploration by MLPA and FISH techniques revealed a 13qter deletion, for both cases. For the first patient, the array CGH (Affymetrix Cytogenetics Whole-Genome 2,7 M Array) identified two terminal deletions of the ring chromosome 13, one of 350 kb and another of 3740 kb in the 13q34 region.

We reported 2 cases of mosaic ring chromosomal 13 associated with deletion of the terminal region of the chromosome 13. This report describe how molecular cytogenetic and array GCH technologies can give more knowledge about the correlation of genotype-phenotype in the mosaic ring chromosome 13 syndrome; and allow to identify the candidate gene responsible for the clinical features of this syndrome.

P03.069
Mosaicism with a normal cell line and a cryptic unbalanced autosomal reciprocal translocation: three new cases

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Mosaicism involving a normal cell line and an unbalanced autosomal translocation are rare. In this study we present three new cases with such a mosaicism, which were detected by Single Nucleotide Polymorphism (SNP) array analysis in our routine diagnostic setting. These cases were further characterized using Fluorescence in situ Hybridisation (FISH) analysis and conventional karyotyping.

The first case is a mentally retarded male who carries an unbalanced translocation in 87% of his cells. The phenotypically normal mother carries the balanced form of the translocation in all her cells. The second case is a phenotypically normal female who has an unbalanced translocation in 52% of her cells. The inheritance could not be determined. The third case is a female referred for Rubinstein-Taybi syndrome who carries an unbalanced translocation in 60% of her cells. Both parents of this case showed a normal karyotype.

The mechanisms that might be responsible for these mosaic karyotypes are discussed. Furthermore, we demonstrate that high-resolution whole-genome SNP array is a powerful tool to reveal cryptic unbalanced translocations and mosaicisms, including the more rare cases.

P03.070
Simultaneous detection of genomic rearrangements in Myelodysplastic Syndromes (MDS) using the Multiplex Ligation-Dependent Probe Amplification (MLPA) assay

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Cytogenetic analysis of the bone marrow is indicated in MDS not only for diagnostic purposes, but also to assess individual prognosis and to plan tailored therapy. Conventional cytogenetic (CC) analysis is performed in clinical practice to detect chromosomal abnormalities. A new method has recently been described for the measurement of the gene/chromosome copy number using genomic DNA: Multiplex Ligation-dependent Probe Amplification (MLPA). The purpose of this study was to compare the results of the MLPA assay with the CC data obtained in a series of 38 MDS patients (M: 28, F:10, median age 70 years, range 44-87). Our study showed a good correlation between the MLPA and CC results (Table I), discrepancies were found in 7 samples (18.5%). MLPA analysis did not detect the presence of a chromosomal (chr.) translocation (sample n°4); a chr. deletion and a chr. translocation (sample n°11); a chr. deletion (sample n°15); several chr. translocations and deletions (sample n°20); a chr. gain (sample n°27). On the other hand, CC analysis did not show a small deletion in 2 samples: a deletion of the MLL gene in 11q23 (sample n°23) and deletion of 7q22 (sample n°37). With CC we also observed a karyotype failure (no metaphases) in 4 samples, while the MLPA assay showed 3 chr. deletions (sample n°14), but no anomalies in the other 3 samples (25, 29, 32). MLPA and CC resulted complementary techniques, MLPA being particularly useful in MDS cases with karyotype failure and for identifying small rearrangements.

Table I

Sample	MLPA	Karyotype
1	No anomalies	46, XX
2	No anomalies	46, XY
3	No anomalies	46, XY
4	Del 5q	46, XY, Del 5q, t(1;12)
5	Del 7	45, XY, Del 7
6	No anomalies	46, XX
7	No anomalies	46, XY
8	No anomalies	46, XY
9	No anomalies	46, XY
10	Del 5q	46, XY, Del 5q
11	Del 5q, Del 11q23	45, XY, Del 11q23, Del 15, der 5 t(5;15) [16] / 46, XY [4]
12	No anomalies	46, XX
13	Trisomy 8	47, XY, Trisomy 8
14	Del 7q, Del 12p, Del 20q	No metaphases
15	Del 11q23	46, XX, Del 11q23 [5] / 46, XX, Del 9q22-23, Del 11q23 [15]
16	No anomalies	46, XY
17	No anomalies	46, XY
18	No anomalies	46, XY
19	No anomalies	46, XY
20	Del5q, Del 12p, Del17p	42~47, XX, Del 1p34 [3], Del 3 [6], t(4;18) [10], Del 5q [18], der 9 [3], Del 12 [10], t(13q;17q) [3], der 15 [4], Del 20 [3], Del 21 [2], Trisomy 22 [3], +m [10] [cp18] / 46, XX [2]
21	Del 20q, Trisomy 21q, Trisomy 19p, Trisomy 8	48, XY, Trisomy 19, Del 20q, Trisomy 21[12] / 48, XY, Trisomy 8, Trisomy 19, Del 20q[8]
22	No anomalies	46, XY
23	Trisomy 11q23 (MLL gene)	46, XX
24	No anomalies	46, XX
25	No anomalies	No metaphases
26	No anomalies	46, XX
27	No anomalies	46, XY,add(11)(p15)[10] / 46, XY[10]
28	No anomalies	46,XY
29	No anomalies	No metaphases
30	Del5q22-33	46, XY, Del5q15-33[8] / 46, XY[7]
31	No anomalies	46, XY
32	No anomalies	No metaphases
33	Trisomia 11	47, XY, Trisomy 11
34	No anomalies	46, XY
35	No anomalies	46, XY
36	No anomalies	46, XY
37	Del 7q22	46, XY
38	No anomalies	46, XY

P03.071
The First Report: The Genotoxic Effect of Nicotine on Chromosomes of Human Embryonic Cells in Culture: the Effect of Cigarette Smoking on the Embryo

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Nicotine can affect embryonic development, leading to the formation of multinucleated blastomeres and polyploidy in blastocysts. Many studies have shown that nicotine interferes with oocyte meiosis and chromosome disjunction. In this regard, in this study we tried to understand the frequency of embryonal chromosome aberrations (CAs) in pregnancies of active or passive smoking mothers. In order to do so, we examined the effect of nicotine sulphate on amniocytes by designing an experimental setting consisting amniocytes grown in nicotine containing medium, study group, and amniocytes grown in a medium which did not contain nicotine (nicotine sulphate), in control group. Here, we provide the evidence that nicotine exposure in vitro (25 ng/ml) (in fetal serum, range 0.5-25ng/ml), has detrimental effects on fetal cells. According our findings, there is a significant difference in terms of CAs between nicotine containing medium and control medium, determined by the $\chi 2$ test (P <0.001). Nicotine containing medium grown cells, totally 577 cells, revealed mainly numerical aberrations (112 cells, %90.3 of all aberrations). Numerical aberrations observed, usually consisted of polyploidy and aneuploidies. We found, most frequently, numerical abnormalities of chromosomes 21, 22, 8, 15 and 20. In conclusion, results of this study confirm an association between prenatal exposure to cigarette smoke and in utero aneuploidies. The nicotine leads to significant direct genotoxic effects in human fetal cells in vitro. Further studies are needed to clarify the modulation of the genotoxicity of smoke causing various genetic polymorphisms.

P03.072
Detection of copy number variants in patients with Noonan syndrome or cardiofaciocutaneous syndrome

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Noonan syndrome (NS) and cardiofaciocutaneous syndrome (CFC) are autosomal dominant congenital anomaly disorders associated with gain-of-function mutations of genes in the RAS/MAPK signaling pathway. Diagnosis is based mainly on clinical grounds characterized by a distinctive craniofacial appearance, heart defects, musculoskeletal and cutaneous abnormalities, and mental retardation. Although molecular genetic testing is available for all known genes, PTPN11, KRAS, SOS1 and RAF1 in NS, and BRAF, MAP2K1, MAP2K2 and KRAS in CFC, overall mutation detection rates are 60% to 70% in both syndromes. To detect pathogenic copy number variants (CNV) for NS and CFC patients who did not have any mutations in sequence analyses of all causative genes, we performed high-resolution CGH-based microarray in 13 NS and 5 CFC patients. Three CFC patients were found to have probably pathogenic CNVs; 3.7 Mb loss in 12q12q13.11, 1.3 Mb gain in 22q11.23, and simultaneous 2 Mb loss in 1p36.33 and 13.0 Mb gain in 17q24.3q25.3. This 22q11.23 duplication has been reported as a recurrent microduplication syndrome. Five CNV regions of unknown clinical significance were observed in 4 NS patients, including gains of GOLGA4, NDUFA4, THSD7A, TPD52L1, EIF3H and FBXO7. In present study, pathogenic CNVs are significantly frequent in CFC than NS patients. This result suggests that patients with CFC-like features can be likely to have large CNVs and need a chromosomal microarray test. And also gene copy changes of unknown significance should be evaluated to validate the clinical relevance.

P03.073
Know how to recognize and appropriately follow-up a recombinant pericentric inversion detected by array CGH

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Nowadays, array-based analysis is the first diagnostic tool to examine patients with mental retardation and congenital anomalies. If a genetic imbalance is observed and confirmed by karyotyping and/or FISH, parents are usually analysed with FISH using probes localized in the unbalanced regions to exclude a balanced rearrangement. This is needed to assess the risk for further children with an unbalanced chromosome complement and to provide prenatal diagnostic options. We present a 20-year old male patient with severe developmental delay and autism. Array CGH analysis revealed a terminal gain of 1.07 Mb of 18p11.32 and a terminal loss of 16.8 Mb of 18q21.33q23. The terminal deletion 18q, also known as DeGrouchy syndrome, explained the phenotype in our patient. At this point, parental chromosome studies were offered but denied. Recently, we received amniotic fluid of the sister-in-law of our proband, because of ultrasound abnormalities.

Chromosome analysis showed an abnormal chromosome 18, suggesting an unbalanced 18;18 translocation with extra 18q-material on terminal 18p. After karyotyping, the prospective father and paternal grandfather proved to be carriers of an inv(18)(p11.32q21.33). Consequently, the fetus and the proband exhibited each a different recombinant chromosome 18. The paternal pericentric inversion would remain unrecognized when FISH with probes from the abnormal regions was performed.
In conclusion, the concomitant gain and loss of the terminal long and short arm of the same chromosome at array diagnostics should alert to a recombinant pericentric inversion. Further karyotyping studies in proband and parents are highly recommended, if necessary combined with metaphase FISH.

P03.074
A new variant of Potocky- Lupsky syndrome in two unrelated patients affected by severe epileptic encephalopathy, detected by Genome Array-CGH

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The duplication 17p11.2 syndrome, associated with dup(17)(p11.2p11.2), is a recently recognized syndrome of multiple congenital anomalies and mental retardation. It is the first predicted reciprocal microduplication syndrome described_the homologous recombination reciprocal of the Smith-Magenis syndrome (SMS) microdeletion (del(17)(p11.2p11.2)(Potocky et al, 2007).
Herein, we report a novel pathologic variant of this syndrome detected by Genome Array-CGH in two non consanguineous subjects with dup(17)(p11.2p11.2),
Array analysis was performed using the BAC Cyto-chips Blue-Gnome platform, at 0.5 Mb median resolution, and revealed the presence of the same BAC duplication in both patients.
The duplicated BAC (RP11-78O7), of about 140 Kb, maps in 17p11.2 and contains an interesting gene, presumably involved in the pathology. The parents did not show the duplication, suggesting that it was a de novo rearrangement.
Cytogenetic and clinical features of subjects with partial trisomy of proximal 17p have been described, mostly in isolated case reports or literature reviews, and non specific and non characterizing findings include developmental delay, mental retardation, and dysmorphic features.
Our patients present a peculiar form of epileptic encephalopathy associated with severe mental retardation and autistic behaviour. Molecular and genetic studies are in progress to define and characterize the rearrangement and for better genotype/phenotype correlation.

P03.075
Subtelomeric fluorescence *in situ* hybridization in clinical cytogenetics: results of analysis of Lithuanian patients

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Background: Subtelomeric fluorescence *in situ* hybridization (FISH) is very multifunctional method in clinical cytogenetics. Usually, subtelomeric FISH is used for detection of subtelomeric rearrangements in patients with intellectual disability (ID). In parallel with detection of subtelomeric deletions/duplications, we have also applied subtelomeric FISH in search or confirmation of other types of chromosome rearrangements: ring chromosomes, translocations, recombinat chromosomes, isochromosomes, mosaics. **Methods:** Analysis has been performed using the set of ToTelVysion™ subtelomeric FISH probes, Nikon Eclipse 80i epifluorescence microscope and LUCIA v1 software. Thirty-eight Lithuanian patients with various clinical features were selected for subtelomeric FISH analysis. **Results:** For detection of subtelomeric deletions/duplications 33 unrelated patients with ID/developmental delay (DD), dysmorphic features and/or multiple congenital anomalies were analysed using subtelomeric FISH. Two subtelomeric aberrations - monosomy of chromosome 1q and monosomy of chromosome 5q were detected. We have chosen subtelomeric FISH as rapid, precise method for confirming balanced translocations in cases where G-banded chromosome analysis results were ambiguous - unclear translocated fragment size or origin. Combining different subtelomeric FISH probe mixtures, we validated two translocations - t(2,12) and t(4,18). Breakpoints of ring chromosomes may be specified by subtelomeric FISH too. We revealed p15 and q35 breakpoints of ring chromosome 5. In one case FISH analysis indicated derivative chromosome 6 which probably has been formed due to pericentric inversion in germline of one of the parents. We confirmed isochromosome X and specified the ratio of the cell clones of mosaicism in patient with Turner syndrome.

P03.076
Genetics Study of Premature Ovarian Failure in Tunisian Population

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Premature ovarian failure (POF) is a common cause of infertility in women and it affects 3 % of females. It is characterized by amenorrhea, hypo-oestrogenism and elevated gonadotrophin levels in women under the age of 40. Known causes include iatrogenic agents that cause permanent damage to the ovaries, such as chemotherapy, radiation therapy and surgery, autoimmune conditions, X-chromosome abnormalities and autosomal genetic conditions. However, few genes have been identified that can explain a substantial proportion of cases of POF.
The present study is interested in the evaluation of the implication of genetic disorders in etiology of POF. Genetic explorations were used and consist to a chromosomal analysis using karyotyping to detect chromosomal abnormalities, interphasic Fluorescent InSitu Hybridization (FISH) to detect 45,X mosaicism, molecular analysis to detect mutation in FMR1 and comparative Genomic Hybridization (CGH) to detect X chromosome microdeletions. 6 chromosomal aberrations were found among the 39 analyzed Karyotypes (15%). Among patients with normal karyotype, interphasic FISH allows us to detect 45,X mosaicism in 12% of cases. FMR1 gene premutation was detected in 16 cases. One microdeletion was detected among 4 patients explored using array CGH (44K Agilent): a 425 kb microdeletion was found on the chromosome 5 of one patient: arr 5p14.1 [28981864-29407781] × 1. The pathological character of this abnormality remains to confirm.
Further genetic studies of POF are needed to identify a genotype-phenotype correlation.

P03.077
Design of diagnostic tests for carrier testing in families with rare microdeletions using molecular genetic methods

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The assessment and carrier testing for rare microdeletions was difficult in the past. Nowadays, using MLPA, FISH and array CGH (aCGH), this task is much easier. We present 4 families with different unique microdeletions. The proband from Family A suffered from developmental delay, obesity and suspected haemophilia B. Molecular genetic testing revealed a deletion of all exons of the *F9* gene. The

phenotype of the boy suggested a larger deletion, and aCGH indeed showed that the deletion affected 5 additional protein-coding genes. A boy from Family B was tested because of familial malignant histiocytosis. A deletion affecting all *SH2D1A* exons was identified using PCR, and aCGH revealed that the deletion also included a part of the *ODZ1* gene. Family C had a history of non-syndromic aniridia. MLPA confirmed a *PAX6* gene deletion in the proband, and aCGH indicated that the deletion also encompassed 3 additional genes. A boy from Family D died of Norrie disease in infancy. PCR identified a deletion of the whole *NDP* gene, and aCGH showed that the deletion included also the *MAOA* and *MAOB* genes. The aCGH results allowed us to select locus-specific FISH probes and to test a total of 14 patients from these 4 families. Eight of them were females at risk in reproductive age, and 3 were shown to be carriers of the microdeletions. FISH is a very reliable method not only for carrier testing, but also for prenatal or preimplantation diagnostics in these families. Supported by CHERISH (EC FP7 223692) and MZOFNM2005.

P03.078
Patients with ring chromosome 11: molecular characterization and review of the literature

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Ring chromosomes are uncommon cytogenetic findings. Most patients carrying a ring chromosome are phenotypically abnormal due to deletions at one or both arms of the chromosome.

Patients with a ring chromosome 11 usually have growth retardation and developmental delay to some degree, while only some of them have clear facial dysmorphic features. Café au lait spots have been described in almost half of the patients.

We present five new patients carrying a ring chromosome 11, with three of the patients belonging to the same family. All patients do have growth problems and café au lait spots. Four of them have microcephaly, while one has a head circumference just within normal limits. Developmental problems are severe in one patient, but do not exist in two other patients.

In four patients a terminal deletion of the short arm of chromosome 11 was demonstrated. In one of them an additional terminal deletion of chromosome 11q was detected, which does not contain any coding genes. The terminal 11p deletion in the familial ring chromosome contains six more genes than in the other patient. For the extra deleted genes no relationship has been reported with growth retardation or microcephaly. In contrast to the other patients, the fifth patient showed a considerable terminal deletion of 14 Mb on 11q, which fits with a diagnosis of Jacobsen syndrome.

The differences in clinical characteristics are most likely due to the differences in size of the deletions these patients have, and the genes involved in these deletions.

P03.079***
BTRC upregulation alone is insufficient to cause SHFM3 - evidence from 4 new cases.

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Split hand-foot malformation (SHFM) is a limb reduction defect, resulting most commonly in absence of the central rays. Severity is variable, often with asymmetric expression, and penetrance may be reduced. It is either isolated or it may be part of a wider, syndromic condition. The aetiology of isolated SHFM is complex and remains to be fully elucidated; it has so far been mapped to five loci: 7q21, Xq26, 10q24, 3q27 and 2q31; however, the critical genes for all except one of these loci have not been identified.

SHFM3 has been mapped to 10q24 and previous studies have shown it to be associated with a duplication of a minimum region of ~325kb, thereby implicating several candidate genes including FBX4, LBX1, and BTRC. FISH and expression studies have previously narrowed the minimal region of involvement to two genes, BTRC and POLL, and have also demonstrated up-regulation of BTRC.

We present 4 new cases with overlapping, different sized duplications of 10q24, detected by array CGH: two cases with SHFM had duplication of FBX4, LBX1, BTRC and POLL, one case without SHFM had duplication of BTRC, POLL and FBX4, and a further case without SHFM had duplication of BTRC and POLL only. Our results therefore indicate that duplication of BTRC is not in itself sufficient to cause SHFM and therefore do not support the hypothesis that over-expression of BTRC alone can lead to split hand foot malformation.

P03.080
SNP-array analysis in prenatal and postnatal molecular karyotyping.

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SNP array represents the unique technique for the identification of cytogenetically undetectable submicroscopic alterations (microdeletions, microduplications) and copy number neutral events (LOH, UPD). During one year (02/10 - 02/11) the whole genome genotyping of 298,649 SNPs in previously (or simultaneously) karyotyped patients was performed using the Illumina HumanCytoSNP-12v2.1. All data were analysed by the GenomeStudiov2010.2 and some of the postnatal samples by the QuantiSNPv2.0. A total number of 251 samples were analysed.

In prenatal diagnosis the array analysis was performed in 99 karyotypically normal foetuses with prenatally identified serious ultrasound anomalies. In 9 cases, healthy parents (n=15) were examined for the assessment of the clinical relevancy of discovered copy number variations. In additional 9 foetuses cytogenetic findings (marker chromosome, de novo translocation, derivative chromosome) were clarified.

In postnatal diagnosis, 82 patients with PMR and normal karyotype and 31 healthy parents were examined. Precise identification of cytogenetically detected chromosomal "balanced" and unbalanced aberrations was performed in additional 15 patients.

In total, 15% (16/108) of foetuses with abnormal ultrasound findings were found to carry clinically relevant CNVs (microdeletions or microduplications). In 12% (10/82) of patients with PMR and normal karyotype clinically relevant CNVs were detected and in additional 17% (14/82) of the CNVs parental samples have not been analysed yet. Chromosomal imbalances were detected in nearly all (13/15) patients with abnormal karyotype. Our data suggest that SNP analysis is clinically significant complementary technique in both prenatal and postnatal diagnosis. In PMR cases SNP analysis should be used as first-choice approach.

P03.081
Somatic mosaicism for chromosome X and Y aneuploidies in monozygotic twins heterozygous for sickle cell disease mutation

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Somatic genetic variation in health and disease is poorly explored. Monozygotic (MZ) twins are a suitable model for studies of somatic mosaicism since genetic differences in twins derived from the same zygote represent an irrefutable example of somatic variation. We report the analysis of a pair of generally healthy female MZ twins, discordant for somatic mosaicism for aneuploidy of chromosomes X and Y. Both twins are heterozygous carriers of sickle cell disease mutation. Genotyping of blood DNA from both twins using Illumina Human 610 SNP array revealed a copy number imbalance for chromosome X in a proportion of cells in one twin. Fluorescent in situ hybridization (FISH) analysis confirmed monosomy X (45,X) in 7% of proband nucleated blood cells. Unexpectedly, FISH analysis of cells from the other twin revealed 45,X and 46,XY lineages, both present in 1% of cells. The

mechanism behind formation of these aneuploidies suggests several aberrant chromosome segregation events in meiosis and mitoses following conception. Our report contributes to the delineation of the frequency of somatic structural genomic variation in normal MZ twins. These results also illustrate the plasticity of the human genome for tolerating large copy number changes in healthy subjects and show the sensitivity of the Illumina platform for detection of aberrations that are present in a minority of the studied cells.

P03.082*

Unrelated hESCs and hIPSCs share a common genomic aberration, detected by molecular cytogenetic techniques.

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Multipotency and proliferative capacity of human embryonic stem cells (hESCs) make them a promising source for basic and applied research as well as in therapeutic medicine. The introduction of human induced pluripotent cells (hiPSCs) holds great promise for patient-tailored regenerative medicine therapies. However, in order to use hESCs and hiPSCs for therapeutic purposes, they must maintain long term genomic stability in culture.

Until recently, G-banding was considered to be the gold standard for chromosomal abnormalities' detection in stem cells. Our goal in this study was to apply high resolution techniques (FISH and CGH), that will enable us to examine stem cells genome with an ever finer resolution.

Analysis of three hESC lines and two hiPSC lines over long term culture revealed a recurrent genomic instability, involving the gain of chromosome 1q. This finding was detected in two unrelated cell lines of different origin and implies that gains of chromosome 1q may endow a clonal advantage in culture.

In addition, aneuploidy rate was evaluated at different passages, using FISH probes (12,13,16,17,18,21,X,Y). Genomic integrity was shown to be maintained at early passages of hESCs and hiPSCs. However, at late passages, we observed higher rates of aneuploidy in hESCs, implying a direct correlation between number of passages and increased aneuploidy rate.

These findings, which couldn't be detected by conventional cytogentic, emphasize the importance of using molecular cytogenetic methods for tracking genomic instability in stem cells. It also demonstrates the phenomenon of genomic instability of hESC lines, which bear a resemblance to tumorogenic processes.

P03.083

Small supernumerary marker chromosome derived from chromosome 3 in patient with severe psychomotor developmental delay and dysmorphism

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We report on 7 years old girl with severe psychomotor developmental delay and dysmorphic features with a de novo mosaic partial trisomy of chromosome 3, involving euchromatic material from 3p and 3q. Dysmorphic features characterised by high triangular forehead with expressed sagital suture, hypertrichosis, wide spaced eyes, ptosis, down-slanting palpebral fissures, strabismus, broad nasal root, very short nose with anteverted nares, short grooved philtrum, triangular mouth, high narrow palate, micrognathia, malformed ears with preauricular sinus on one of sides and skin lesions typical for chromosomal mosaicism: streaked, whorled and mottled areas of hypopigmentation on trunk and limbs. CT scan showed corpus callosum agenesis and hydrocephaly, X-ray: abnormal feet position.

Conventional cytogenetic analysis revealed female karyotype with presence of supernumerary marker chromosome in 85% of analyzed cells (47,XX,+mar [17]/ 46,XX [3]). The origin of supernumerary marker chromosome was identified by FISH using whole chromosome painting probe for chromosome 3. It demonstrated that marker chromosome derived from chromosome 3 resulting in partial duplication of proximal 3p and q chromosomal regions. The supernumerary chromosome involves more then 30 genes, including EPHA3, POU1F1, ROBO1, ARL13B, which play role in developmental processes. Overdosage of these genes could affect normal development because most embryonic developmental processes depend on strictly balanced levels of proteins. We hypothesize that supernumerary chromosome resulting in extra gene copies lead to severe psychomotor developmental delay and facial dysmorphism.

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P03.084

Refined molecular cytogenetic characterization of the breakpoints of small supernumerary marker chromosomes derived from chromosome 15

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Many rearrangements may occur in the imprinted chromosome region 15q11->q14, which is known for its instability due to the presence of repeated DNA elements. This region harbours six common sites that mediate chromosomal rearrangements (BP1 to BP6). Supernumerary marker chromosomes (SMC) originating from chromosome 15 are the most common SMCs. Genotype/phenotype studies have not been able, to date, to show any correlation between type and size of clinically relevant SMC(15)s and the degree of severity of the clinical spectrum.

We report 6 new cases of patients with a SMC(15) that were characterized by molecular cytogenetics (Multicolour FISH) and array CGH techniques, followed by microdissection, for the evaluation of the exact genetic content, correlating clinical features with SMC(15) sizes. One case is a small SMC(15)s and without clinical features and/or development delay. The other 5 cases are large SMC(15)s with the Prader-Willi/Angelman syndrome critical region, all having clinical features associated. Of these large SMC(15)s described, two cases are from group B, having symmetrical pattern corresponding to BP3 (BP3:BP3). The other 3 cases have large SMC(15)s from group A, showing an asymmetrical pattern with distal margins at the known breakpoint BP4.

A full characterization of SMC(15) by array CGH techniques is essential to establish with more precision breakpoints position and size of duplicated segments. Array CGH analysis, after microdissection of the marker, showed to be a powerful approach to detect the duplication and its extent.

P03.085

Genomic microdeletions in patients with syndromic mental retardation diagnosed by molecular cytogenetic and CGH-array analysis.

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Non allelic homologous recombination is supposed to generate cryptic microdeletions as well as microduplications, in the field of mental retardation numerous well-known microdeletion syndromes have been described. We explore twenty five patients with mental retardation and known to be the target of microdeletion (Table 1). The patient's chromosomes were characterized by fluorescence in situ hybridization (FISH) with probe which contains two or three locus region-specific probes. Hybridizations were performed following manufacturer's instructions (**Vysis®).** In this series, we found a 7q11.23 deletion removing the *ELN* gene in four patients with congenital cardiopathy,

profound MR and, retrospectively, craniofacial feature of Williams-Beuren syndrome, a 17q13.3 deletion in a patient with a lissencephaly revealed by magnetic resonance imaging, a 15q11-q13 deletion in a boy with craniofacial feature of Prader-Willi syndrome and a *UBE3A* deletion caused an Angelman Syndrome in a boy with microcephaly, severe epilepsy, language delay and ataxia. For the eighteen normal patients, we explore the DNA with The 44000 Agilent® oligonucleotides. Array-CGH analysis of the 18 patients showed a deletion of 200 Kb in 2q23.1 in a patient presenting similar pseudo-Angelman phenotype, this deletion will be verified with BAC clone RP11-659J19.
The systematic screening of patients with syndromic MR, using FISH and CGH-array diagnoses, should facilitate the estimation of microdeletions frequency in MR population and the characterisation of novol syndrome

Table 1. Diagnosed patients with fluorescence in situ hybridization

Number of diagnosed patients	Syndromes	Localization	Targeted gene
4	Williams-Beuren	7q11.23	*ELN*
3	Prader -willi	15q11-q13	*SNRPN*
9	Angelman	15q11-q13	*UBE3A*
4	Digeorge	22q11.2	*TUPLE1*
1	Wolf-Wirschhorn	4p16.3	*WHSC1*
1	Cri-du-chat	5p15.2	*D5S23 , D5S721*
1	Miller-Dieker	17p13.3	*LIS1*
1	Sotos	5q35	*NSD1*
1	Smith-Magenis	17p11.2	*RAI1*

P03.086
Molecular analysis of telomere length in subjects with intellectual disability

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Telomeres are non-coding regions of DNA that maintain genomic stability. Telomere length decreases with cell divisions despite telomerase which consists of two subunits: TERT (the catalytic unit) and TERC (the RNA component). This process is related directly with cellular and individual aging. The aim of this study was to analyze differences between telomere length in people with Down Syndrome (DS), people with cerebral palsy (CP) and people without intellectual disability. We collected saliva samples from 122 subjects with DS (with a mean age of 34,61) 59 with CP (mean age: 30,85) and 115 without intellectual disability (mean age: 47,65). Genomic DNA was extracted from buccal cells and telomere length was measured by qPCR relative comparative. In addition, we analyzed two polymorphisms associated with lower activity of telomerase: TERT-1327C>T (rs:2735940) and TERC-63G>A (rs:2293607). The relative telomere length and the polymorphisms analysis are shown in table. The analysis of the telomere length in subjects over and under 40 years old, shows significant results in DS and CP (p=0,021 and p=0,050 respectively) but not in controls (p=0,202).

	Telomere length	**TERT (-1327C>T)**	**TERC (-63G>A)**
Probands Vs Controls	P<0,001	P=0,152	P=0,055
DS Vs Controls	P<0,001	P=0,316	P=0,102
CP Vs Controls	P<0,001	P=0,145	P=0,181
DS Vs CP	P=0,952	P=0,711	P=0,898

Our data demonstrate premature biological aging in people with DS and show, for the first time, that people with CP has chromosomal telomeres smaller than people without intellectual disability. The lack of correlation between TERT-1327C>T and TERC-63G>A polymorphisms and telomere shortening suggests the existence of other biological factors involved in the process.

P03.087
Constitutional tetrasomy 18p in a female child - a new case report

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Tetrasomy 18p is a very rare chromosomal disorder and is the result of a spontaneous mutation early in embrionic development in most of the cases. It has a prevalence of 1/140000 live births and affects both genders equally.
In this paper we reported a de novo tetrasomy 18p in a 3 months old female dysmorphic child. The clinical features were distinctive, with a particular facies, strabismus, neonatal hypotonia and microcephaly. A small metacentric marker chromosome has been identified after standard cytogenetic analysis, without recognized parental origin of the supplementary genetic material. The child's parents were also tested and their karyotype results were normal.
The characterization of the marker chromosome using only conventional cytogenetic methods was difficult. Consequently, the marker was evaluated by molecular cytogenetic techniques. Fluorescence in Situ Hybridization (FISH) analysis was the first step of diagnosis. Two types of DNA FISH probes were used in our study to describe the structure of the marker: a probe for the centromeric region of chromosome 18 and probes for the subtelomeric regions of chromosome 18. A more detailed description of the identified isochromosome 18p was performed using MLPA technique.
Our patient was compared with other published cases with tetrasomy 18p.

P03.088***
Balanced translocations and inversions in the genome of Finns

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Finland is acknowledged of its high standard of clinical medicine and in the disease gene hunt of its founder populations. What is perhaps not so well recognized is that Finland has probably the most comprehensive health registers and records: hospitalizations, surgeries, chronic diseases, cancers and prescriptions etc. have been filed for decades.
Relying on this infrastructure we have gathered clinical information from all distinct hospitals and laboratories on all reciprocal balanced translocations and inversions identified in Finland, and are currently constructing a national database (www.fintransloc.org). By analyses of the medical records and by searches of additional national registers, we are obtaining novel information of not only monogenic traits with unidentified mutations, but also of multifactorial traits associated with any given chromosomal abnormality. Moreover, we believe that such a database will greatly assist genetic counseling efforts and scientific collaborations.
To date, we have surveyed 2575 hospital or laboratory contacts involving translocations or inversions consisting of 572 families plus singletons. Some 25 families associating with interesting abnormal phenotypes are selected to investigate the breakpoints' potential effect on the phenotypes. A nationwide systematic sample collection of DNA, RNA and cells with so far over 80% coverage of participants was launched in Aug 2010, and we are breakpoint mapping three pilot families providing presumed shortcut in the identification of their predisposing disease genes.

P03.089
Two rare modes of paternal malsegregation of a translocation t(11;13)(q25;q14) in his two sons

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We report a rare case of malsegregation of paternal balanced chromosomal reciprocal translocation t(11;13) in his two sons. The family was seen in genetic counseling for moderate psychomotor delay associated with dysmorphy and immunity deficiency for one son (son 1) and strong psychomotor delay associated with dysmorphy and cardiopathy for the other son (son 2).

Conventional cytogenetic studies show a paternal karyotype with balanced reciprocal translocation t(11;13)(q25;q14) and a maternal normal karyotype. For the son 1, karyotype followed by 105K-Agilent aCGH study revealed partial trisomy of 11q (780Kb) associated with partial trisomy of 13q (28Mb). The son 2 carried a partial monosomy of 11q and partial trisomy of 13q.

This is an unusual occurrence of paternally inherited unbalanced of (1) double partial trisomies (11q;13q) resulting from 3:1 segregation, and (2) partial trisomy 13q and monosomy 11q from adjacent-2 segregation, both in paternal meiosis. Indeed these two modes of malsegregation, rather described in female meiosis, are uncommon in male meiosis and implicate the acrocentric 13 chromosome. Differences in severity of clinical features of both sons are observed. The son 2 developed a more serious phenotype resulting particularly from the monosomy 11qter described associated with Jacobsen syndrome. Clinical features of the son 1 will be discussed in relation with the types of anomalies, the genes localized in the duplications or at their breakpoints with genotype/phenotype correlation and will be compared with previous reports. This report emphasizes the added importance of array-CGH to analysis of offspring born to chromosomal reciprocal translocation carrier parents.

P03.090
Unbalanced transmitted translocation t(3;20) associating epilepsy, behavioral disorders, and severe mental retardation: case report

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We prezent a two year-old boy referred for genetic investigations due to epilepsy and behavioral disorders with heteroagresivity. In addition, he exhibited dysmorphic features (large ears, bilateral preauricular pit, long eyelashes, hairy eyebrows, depressed nasal bridge, bulbous nose, thick lips, ogival palate), severe mental retardation. Congenital heart and renal defects, left umbilical and inguinal hernia were detected.

Classical and molecular cytogenetic investigations were performed on peripheral blood cultures by GTG-banding and FISH standard protocols.

Cytogenetic analysis showed trisomy for the region 3q26.2-qter and monosomy for 20q13.3-qter as a result of an unbalanced translocation. The rearragement was inherited from his clinically healthy mother, who presented a balanced translocation: t(3;20)(q26.2;q13.3). The results were confirmed with BAC-probes FISH.

Both anomalies, duplication of distal 3q and deletion of distal 20q, were previously reported and some of the associated clinical features are also present in our patient. Moreover, trisomy 3q2 is known as a rare syndrome with variable phenotype, depending on the size of the duplicated fragment and the involvement of other chromosomal regions.

The regions involved in our patient chromosomal rearrangement include several genes known to act in nervous system development, such as: NLGN1 on 3q region and ARFGAP1, CHRNA4, KCNQ2 on 20q region.

By our knowledge, this is the first reported unbalanced translocation involving 3q and 20q regions, further contributing to a better understanding the role of these two chromosomal abnormalities in producing of a such complex phenotype.

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P03.091
Double partial trisomy 14q and 22q due to maternal translocation t(14;22)(q23.2;q13.32) with unusual clinical findings

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Trisomies of two autosomes in the same karyotype, even parcially, are very rare in livebirths. We report on 18-month-old male presenting partial proximal trisomy 14q and partial distal trisomy 22q resulting from a maternal translocation. At 13th day of life, he started feeding difficulties, failure to thrive and oliguria. A ventricular septal defect and West syndrome had been diagnosed. At 13 month-old was observed neuropsychomotor delay, hypotonia, microphthalmia, hypertelorism, broad nasal bridge, low-set ears, short neck and phimosis. A cerebral MRI disclosed callosum corpus dysgenesis and ultrasonography showed abnormal rotated kidneys. Neurophysiologic studies were suggestive of auditory dysfunction. Radiographic studies showed toes anomalies. Cervical and lumbar CT studies demonstrated C1, C2, C3, L5 and sacral dysraphism besides T4 and T5 lateral processes fusion. EEG disclosed hemi hypsarrhythmia. He is under anticonvulsivant and gastroesophageal reflux therapy. Cytogenetics analysis showed karyotype 47,XY,t(14;22)(q23.2;q13.32)mat,+der(14)t(14;22). The FISH performed on metaphases with commercially available N25/SHANK3 probes confirmed the karyotype.

To our knowledge, the patient is a first case with partial proximal trisomy 14q and partial distal trisomy 22q. The patient is resultant of alternate 2:2 meiotic segregation containing t(14;22)(q23.2;q13.32) in his chromosome complement. A second event occurred probably in meiosis II with no-disjunction leading to extra der(14)t(14;22). The phenotype includes characteristics of trisomy 14 mosaicism, partial trisomy of proximal 14q, and trisomy of 22q13.3-qter. Moreover, some aditional features are presented on this patient: low-set rotated kidney, skeletal abnormalities with vertebral anomalies, and West syndrome.

P03.092
True trisomy 2 mosaicism in CVS and amniocytes: a new case

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True trisomy 2 mosaicism is a very rare event. Few cases with variable phenotypic outcome are reported in the literature. We report a new case of prenatally diagnosed mosaic trisomy 2.

Transabdominal chorionic villus sampling was performed at 15 weeks' gestation (WG) on a 37-year-old woman because of an increased risk of aneuploidy (1/40). Fetal ultrasound revealed hyperechogenic bowel, oligoamnios, hypotrophy and moderate ventriculomegaly. Among the 21 cells observed from the direct preparation, 19 showed 47,XX,+2 karyotype. Trisomy 2 was also present in almost all cells observed from cultures as well as with conventional cytogenetics and with in situ hybridization. At 17 WG, ultrasound revealed only hypotrophy. Follow up amniocentesis was performed at 18 WG and showed on in situ preparations, a mosaic karyotype mos 47,XX,+2[14]/46,XX[86]. At 21 WG, ultrasound displayed hyperechogenic bowel, retrognatism and hypotrophy. Parents opted for termination of pregnancy. No trisomic cells were observed at cordocentesis. At post mortem examination, there was moderate intrauterine growth retardation, no dysmorphy and no malformations. Nevertheless, examination of the brain showed pachygyria, micro-polygyria and focal neuronal heterotopia.

This case is compared with other reported cases of the literature and the follow-up of prenatally diagnosed mosaic trisomy 2 is discussed.

P03.093
Combine application of different cytogenetic techniques for characterization of dicentric X mosaicism in case with Turner syndrome

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Phenotype of Turner syndrome includes short stature, ovarian failure and other somatic stigmata. It is associated not only with X chromosome complete monosomy, but also with its mosaic and partial monosomy forms. Here we describe a case with Turner-like phenotype in 19-year-old female. Initial chromosome analysis of cultured leukocytes revealed female karyotype with derivative X chromosome in all metaphases, where existence of extra chromosomal material was predicted.

However, Afymetrix SNP 6.0 array data of peripheric blood DNA shown only a 48Mb terminal deletion of Xq22.3-qter region (chrX:106762523-127573136; chrX:127574179-154887041) and no gain of X nor other chromosomal material, which contradicted the karyotype picture. We assumed that der(X) is a dicentric chromosome derived from two Xpter-q22.3 chromosomal materials represented in mosaic form in peripheral blood cells.

Further FISH application on cultured leukocytes using centromere probe SE X (Kreatech) confirmed that derivative X chromosome has two X centromere material and thus is dicentric - 46,X,dic X(pter-q22.3). Despite the fact that der(X) was presented in all metaphases in addition to normal X chromosome, in 30% of interphase cells we detected only two signals. In buccal mucosa cells mosaicism was detected with following ratios: 70%, 17% and 13% of cells with one, two and three signals, respectively.

Thus, neither conventional karyotype study nor SNP array didn't find cells with 45,X. Only interphase FISH on buccal mucosa cells discovered monosomy X which could explain our patient phenotype. Our results demonstrate the importance of different tissues study by conventional and molecular cytogenetic techniques to reveal true mosaicism.

P03.094
A de novo interstitial deletion del(2)(q36.1) including PAX3 gene in a girl with hearing loss and subtle dysmorphic features.

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Waardenburg syndrome (WS) is an autosomal dominant syndrome which is reported to be the most common form of inherited congenital deafness, with a prevalence of 1:42.000. It is strongly heterogenous with wide spectrum of phenotypes, which resulted in identification of 4 forms of this syndrome. The most characteristic dysmorphic features are pigmentary abnormalities of the hair, iris heterochromy and congenital sensorineural hearing loss. Types 1 and 3 of WS are mainly caused by mutations in the PAX3 gene. Deletions of this gene are less frequently reported.

A 16 years old girl was referred for genetic counseling due to hirsutism, hearing loss, hypertelorism and minor phenotypic features. She was a second child of healthy, unrelated parents.

The dysmorphy included wide set eyes with brilliant blue irides, synophrys, shortened eyelids, hypoplastic alae nasi, low set ears, large hands and feet and profound hearing loss. Her intellectual development was normal.

Routine cytogenetic analysis revealed normal karyotype (46,XX). Molecular screening of GJB2 gene excluded its most common mutation (del35G).

Array CGH analysis with Agilent SurePrint 3G Human CGH Kit (4x180K platform) showed an interstitial deletion of long arm of chromosome 2 [del(2)(q36.1)] of 860 kb, including the PAX3 gene. Subsequent analysis performed in parents revealed no rearrangements in the same region, which led to the conclusion that deletion present in the child was de novo.

To confirm these results, FISH analysis with PAX3 breakapart probe was performed.

Results of our study indicate, that WS can also be caused by chromosomal microdeletions involving PAX3 region.

P03.095
Report of a patient with distal Williams-Beuren deletion.

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A 7-years-old boy was referred to the genetic consultation given psychomotor delay, learning difficulties and dysmorphism. He presents behavioral disorders as depression, impulsivity, hyperactivity and relationships difficulties with other children. EEG shows epilepsy. He uses short sentences but with pronunciations difficulties. Developmental quotient was evaluated at 63 (Terman-Merrill). Minor dysmorphic features were observed with upslanting palpebral fissures, strabismus, mild retrognathia and protruding upper lip with long philtrum. Growth parameters at 7-years are in the normal range.

CGH analysis showed a 2.015 Mb deletion distal to the Williams-Beuren deletion. The deletion is maternally inherited. The family belongs to underprivileged socio-cultural environment with relatives presenting learning difficulties and intellectual disabilities. Given this blurring familial situation, this deletion was initially considered as being of unknown clinical significance. Furthermore polymorphic CNV were reported in this region.

Two publications (Edelmann *et al.*, 2007 ; J Med Genet **44**:136-143 and Ramocki *et al.*, 2010 ; Am J Hum Genet **87**:857-865) reported deletions or duplications distal to the Williams-Beuren deletion. These patients exhibit variable expression of epilepsy, learning difficulties, intellectual disabilities (mild to severe developmental delay or intellectual disability with autistic features in some patients) and/or neurobehavioral abnormalities (inattention, hyperactivity, impulsivity and aggression including self-abusive behavior and depression). The majority of the patients have a deletion inherited from a normal or less affected parent. HIP1 and YWHAG genes are also deleted in our patient. However the inheritance from normal relatives, strongly suggests an incomplete penetrance of this deletion which preclude a unambiguous genetic counseling.

P03.096
Williams-Beuren syndrome (WBS) atypical phenotype due to rare familial inversion inv(7)(p10q11.23) in mother and her child: fluorescence in situ hybridization (FISH) analysis of rearrangement breakpoints

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WBS is a neurodevelopmental disorder characterized by distinct facial features, supravalvular aortic/peripheral pulmonary stenosis, mental retardation and typical behavioural features, growth deficiency, infantile hypercalcemia. Typical WBS is characterised cytogenetically by hemizygous 1,55-1,83 Mb deletions including 26-28 genes at chromosomal segment 7q11.23. Diagnostic test for the standard microdeletions detection for classical and atypical WBS is FISH analysis using DNA probes bearing elastin gene locus ELN.

We present a rare family case with atypical WBS phenotype in mother and her 1,2-years old daughter who both had a cytogenetically visible (GTG about 550-600 bands) apparently pericentric inversion. Molecular cytogenetic analysis of chromosome 7 breakpoints using DNA probes for WBS critical region 7q11.23, specific centromeric 7alpha-satellit probe and set of BAC-probes RP4-771P4, RP11-467H10, RP11-343J14 revealed the inv(7)(p10q11.23) in mother and in her child. FISH showed that one breakpoint of inversion is localised within the centromere 7p10 dividing it into two alphoid segments and making abnormal chromosome 7 as pseudodicentric. The second breakpoint was mapped in segment 7q11.23 more distal regarding to centromere of WBS single copy region and distal to the low copy repeats (LCR) sequences B mid, A mid, B tel, A tel and C tel blocks. ELN gene was not disrupted or deleted, therefore supravalvular aortic/peripheral pulmonary defects were absent in the mother and

child, though both of patients had distinctive WBS facial features. We present the clinical data of cases and hypothesize that balanced intrachromosomal rearrangement induces position effect in WBS critical region neighbouring with centromeric sequences.

P03.097
A balanced translocation t(5 ;7)(q32 ; q11.22) disrupting the WBSCR17 gene in a patient with incomplete Williams-Beuren syndrome

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The molecular characterization of balanced chromosomal rearrangements associated with an abnormal phenotype (6% of balanced chromosomal rearrangements), has been a successful approach for the identification of disease-causing genes.
Here we describe the breakpoint mapping of a de novo balanced translocation t(5;7)(q 32;q 11.22) in a male patient presenting with a psychomotor delay and moderate mental retardation, associated with facial anomalies observed in Williams-Beuren syndrome (WBS). He has neither cardiovascular malformations nor abdomino-renal anomalies.
Breakpoint mapping on chromosome 7 with FISH, using BACs clones showed that WBSCR17 gene (Williams-Beuren syndrome critical region 17) was disrupted. However, breakpoint characterization on chromosome 5 was not achieved yet. For this we will perform a new technology called array painting, which could map the position of translocation breakpoints more precisely either on chromosome 7 and 5 in a single experiment.
Array-CGH (Whole genome Tiling NimbleGen :Hg18,72K) confirmed that the translocation was balanced, and showed the absence of other unbalanced rearrangements which could explain the phenotype.
These results strengthened the hypothesis that WBSCR17 is responsible for the neuropsychological findings in our patient, in line with its known implication in brain development. However, its implication in facial anomalies is less likely. For this the fine mapping of breakpoints on the two translocated chromosomes by array painting will help us to better understand the genotype / phenotype correlation in our patient.

P03.098
Rare case of 46,X,idic(X)(p11.2)/46,XX mosaicism in an autistic child with developmental delay: a karyotypic marker of X chromosome isodisomy and an X-linked mutation

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Isodicentric chromosomes X are frequent in females with Turner syndrome demonstrating 45,X mosaicism. In contrast, the case reported herein represents mosacism manifesting as 46,X,idic(X)(p11.2)/46,XX without Turner syndrome phenotype and without a cell with 45,X karyotype. In average, 46,X,idic(X)(p11.2) was detected in 10% of cells. The latter was found using multiprobe FISH on 350 metaphase plates and 1000 interphase nuclei. The structure of idic(X) was determined by FISH, as well. BrdU replication banding showed consistent inactivation of idic(X) in all affected cells. Phenotypic features referred to autistic and aggressive behavior (including self-mutilation), severe developmental delay, lack of speech, mild microcephaly, abnormal pigmentation and mild facial dysmorphisms. Taking into account the severity of the phenotype and the amount of abnormal cells, we came to a conclusion that the index case is likely to be associated with an X-linked mutation and X chromosome isodisomy. To support this hypothesis, we have analyzed length of (CGG)n and (CAG)n repeat expansions of *FMR1* and *AR* genes, respectively, using methyl-sensitive restriction and QPCR, which showed the same parental origin of chromosomes X. Therefore, X chromosome isodisomy and an X-linked mutation are most likely to explain phenotypic manifestations in the index case. Looking through the available literature, we concluded that this is the first reported case of mosaicism involving idic(X)(p11.2) without 45,X cell line. Finally, this case shows that such mosaicism is a karyotypic marker of X chromosome isodisomy and X-linked mutations as well as supports association of autism with mosaic X chromosome aneuploidy.

P03.099
A case of the de novo large duplication of 3pter-p13 and deletion of Xpter-p11.3 in a patient with clinical features of Turner syndrome

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Phenotypic manifestations of unbalanced X;autosome translocations in female carriers are usually modulated by X-inactivation. Spreading of inactivation on the autosome fragment can provide gene silencing. But there are controversial data about continuous or discontinuous mode of this process. We report a case of the de novo unbalanced translocation between chromosome X and 3 in a girl with major clinical features of Turner syndrome, gonadal dysgenesis and normal intellectual development. Patient is a 16-year-old girl from first pregnancy. She had a short stature (142 cm), short webbed neck, low frontal and nuchal hair level, slight epicanthus, synophrys, alae nasi hypoplasia, rotated auricles with adherent earlaps, macrostomia, high arched palate, flat chest, pectus excavatum, hypertelorism of nipples, forearm valgus, hypoplasia of little fingers, camptodactily of 3, 4, and 5 fingers, short big toes, sandal fissurae, hirsutism, hypertrichosis, uterus and ovarian aplasia. The patient had a pronounced myotonic syndrome. The karyotype was 46,X,der(X)t(X;3)(p11.3;p13). Both parents had normal karyotype. The presence of the almost entire additional short arm of chromosome 3 had no visible effects because of suspected X inactivation. Analysis of allele polymorphism in the *AR* gene by *Hpa*II digestion has confirmed a 100% skewed X-inactivation. However, the spreading of gene silencing was probably discontinuous because of the presence of camptodactily, which was reported previously in patients with partial trisomies 3pter-p23. Short neck and pectus excavatum were also observed in cases of dup(3)(pter-p25). To our knowledge this is a first report of large duplication without major clinical malformations of trisomy 3p syndrome.

P03.100
A 17q24.2-q24.3duplication in a patient with hypertrichosis and gingival hypertrophy

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A 6-year-old boy was referred for evaluation of generalized hypertrichosis that was marked at birth. Furthermore he presented gingival overgrowth that began during early childhood causing hoarseness and difficulty in speaking and in chewing.
Array experiments were performed with a custom made array based on Agilent Technologies, called KaryoArray®. The array comprised specific probes covering all microdeletion and duplication syndromes, telomeres and peri-centromeric regions and also probes of backbone. The average density of the probe coverage is 43 kb. This focused oligonucleotide chip covers more than 350 clinically relevant regions of genomic imbalance.
KaryoArray® identified a copy-number gain of 30 oligo probes spanning 1.579 Mb at 17q24.2-q24.3 (chr17:64150313-65728881-NCBI_Build36). Also array CGH analysis was performed on both parents and neither parent was found to carry a duplication. The duplication identified in our case encompasses 8 genes: ABCA8, ABCA9, ABCA6, ABCA10, ABCA5, MAP2K6, KCNJ16 and KCNJ2.
In one previous reported patient with duplication in chr17:64306843-65736803 the authors points MAP2K6 as a candidate for hypertrichosis and gingival hypertrophy because activating mutations in components of the RAS-MAPK pathway can result in a group of phenotypically overlapping genetic syndromes with several dysmorphic features common to the patients reported in their paper. Altered expression

of the dosage-sensitive genes caused by copy-number mutations has traditionally been viewed as the general pathogenic mechanism for genomic disorders. and Congenital hypertrichosis has been documented in several cases with chromosome 17q trisomy, suggesting that hair overgrowth can result from increased function of the gene(s) on chromosome 17q.
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J03.01
A normal man with uncommon t(15;16)

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Reciprocal translocations are usually an exchange of material between nonhomologous chromosomes. Estimates of incidence range from about 1 in 500 to 1 in 625 human newborns. whole-arm translocations result from centric fusion of two chromosomes. Such translocations are usually harmless and may be found through prenatal diagnosis. However, carriers of balanced reciprocal translocations have increased risks of creating gametes with unbalanced chromosome translocations leading to miscarriages or children with abnormalities.Most balanced translocation carriers are healthy and do not have any symptoms. But about 6% of them have a range of symptoms which may include autism, intellectual disability, congenital anomalies, or subfertility. In contrast, patients with unbalanceed whole-arm translocations have abnormal phenotypes.

We report a normal couples which referred by the gynecologist for genetic counseling just because the wife had irregular menstrual cycles. They had a normal son as well.This couples were interested to participate in one of our research project as a normal control. Cytogenetic studies were performed on lymphocyte cultures using G-banding techniques. It was interesting that all the mitoses of the wife showed a reciprocal whole-arm translocation between chromosomes 15 and 16, which according to literatures is very uncommon.

The karotype of wife was: 46,XX,t(15;16)(p10;p10).

Chromosomal investigation on her parents, her husband and her son revealed normal karyotypes.

J03.02
Chromosomal evaluation in couples with reproductive disorders - retrospective study of a selected group of 266 couples

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Chromosomal evaluation in couples with reproductive disorders - retrospective study of a selected group of 266 couples (Abstract): Reproductive Disorders (RD), manifested by the biological inability to conceive (primary sterility) or inability to carry a pregnancy to full term (infertility), affect 10-15% of reproductive-aged couples. The genetic etiology of RD is represented, in the majority of cases, by the chromosomal abnormalities. Aim: To retrospectively analyze the karyotype results in a selected group of couples with RD. Material and methods: The present study was performed in 266 couples with RD: 80 (30,07%) with primary sterility (ST), 149 (56,01%) with Recurrent Spontaneous Abortions (RSA) and 37 (13,90%) with Stillborn Children (SC). A GTG-banded karyotype was performed on both parteners of each couple. Results: We identified a chromosomal abnormality in 43 individuals (16,16%): 20 cases (7,51%) with ST, 13 cases (4,88%) with RSA and 10 cases (3,75%) with SC. The affected partner was female in 23 cases (8,64%) and male in 20 cases (7,51%). A X chromosome (numerical or structural) abnormality was detected in 18 cases (6,76%), most frequent X chromosome monosomy mosaicism in female and trisomy XXY in male; a balanced structural chromosomal abnormality (BSC) was detected in 23 couples (8,64%); in other two males with ST, the karyotype result was 46,XX. Conclusions: The results of our study are similar to other reported studies and underline the major etiologic role of chromosomal abnormalities in RD and the importance of chromosomal analysis for the etiologic diagnosis and genetic counselling of these patients.

J03.03
Familial Pericentric Inversion of Chromosome 12

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Chromosomal inversion is a balanced rearrangement defined as two breaks with reinsertion of the chromosome after the segment rotates 180^0. The frequency of chromosome inversions is estimated approximately 0.13 in 1,000 in the general population. Most of the individuals carrying inversion are phenotipically normal. Although rarely seen, pericentric inversions are among the most common constitutional chromosome rearrangements and typically involve chromosomes 1, 3, and 7. Pericentric inv (12) is extremely rare, with only 25 reported families in the literature. The common constitutional inversions are the pericentric p11q13 and p13q13. The inv (12) is difficult to detect and may be misinterpreted as a partial deletion by routine cytogenetics. Pericentric inversion of chromosome 12 can be associated with epilepsy, mental retardation and hematological malignancies. Here, we report on a familial inv (12) (pter→p11::q13→p11::q13→qter) in father and two siblings who did not have any clinical manifestations. The mother's chromosomes were normal in blood lymphocytes. Karyotyping was routinely performed by G-banding by using trypsin-Giemsa staining technique. In the light of the findings of our family, we will discuss the literature about pericentric inv (12).

J03.04
Numeric Chromosomal Changes in Acute Leukemia

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Acute myelocytic leukemia (AML) and Acute lymphoblastic leukemia (ALL) is characterized by a variety of numerical and structural chromosome aberrations. Gains and losses of whole chromosomes occur frequently in AML and ALL, both as solitary changes, usually found at diagnosis, and as additional aberrations in later disease stages. 120 cytogenetic analyses of patients with leukemia with ages between 6 month -73 years have been performed in the Cytogenetic laboratory of the University of Medicine and Pharmacy Victor Babes Timisoara during 2004-2010. The cytogenetics diagnosis is a prognostic factor with the capacity to predict the remission rate, the length of the remission and the survivorship period, regardless of the haematological, immunological and clinics parameters. For the confirmation of previous data, CGH may provide useful information regarding the nature of genomic aberrations that take place in cases with complex karyotypes.

J03.05
Report of five de novo translocation Down's syndrome

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The frequency of Down's syndrome in the most populations is around one in 700-800 live births and shows different karyotypes. Trisomy 21 is the main cause of approximately 95% of observed Down's syndrome, with 88% coming from nondisjunction in the matenal gamete and 7% coming from nondisjunction in the paternal gamete. Robertsonian translocation and isochromosome of the long arm of chromosome 21 are the cause of 2-3% of observed cases of Down's syndrome. It does not show the maternal age effect, and is just as likely to have come from fatherts as mothers. The remaining 1-2% are mosaicism.In about three-fourths of translocation Down's syndrome, neither parent is a carrier, and a mutation in the germ cells of one parent has caused the translocation. The causes of these mutations are unknown.

We report five infants (three boys and two girls) from the five different families, all revealed typical clinical features of the Down's syndrome. The parental age of the five cases were between 20-30 years old. Cytogenetic study were carried out, using the standard banding techniques, for the cases and their parents. The karyotypes of the three cases were: 46,XY,der(21;21)(q10;q10),+21 and the other two cases showed: 46,XX,der(14;21)(q10;q10),+21. Chromosomal analysis of the parents of the five infants were normal.

J03.06
Cytogenetics studies im mild Mental Retardation

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This study was developed to review the clinical criteria for karyotyping studies in mild mental retardation (MR) patients.

We created 2 patients groups: a) in the first we had 107 patients with mild MR and we do karyotype analysis in 34 selected using the classical clinical criteria (2 ou more malformations and /or dysmorphic features associated) and only in 3 patients (2.8%) we detected chromosomal anomalies (all sexual chromosomal aberrations: 46, XX/45,X; 47, XYY; 48, XXYY). B) in the second group we had 187 patients and we did karyotype analysis in 172 of them independently of the patient had malformations and / or dismorphic features and we detected 10 patients (5.8%) with chromosomal aberrations [6 of them with sexual chromosomal aberrations: 47, XXX (2); 47, XYY; 48, XXXY; 46, XX/45, X; and 4 with autosomal aberrations).

Our work suggested, similar to others authors, that in mild MR the karyotype may be indicate in all patients, independently of the presence of malformations and / or dysmorphic features.

J03.07
Peculiarities of replication of 1qh, 9qh, 16qh and Yqh regions of human chromosome from chorionic villi and embryonic tissues.

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The aim of study was analysis of replication of 1qh, 9qh, 16qh and Yqh regions of human chromosome on semi-direct preparations from chorionic villi samples (CVS) and embryonic tissues (ET). CVS were incubated with BrdU during 12, 24, 36, 48, 72 hours, and ET - 12, 24 hours.

Bright red fluorescence of 1qh, 9qh, 16qh and Yqh regions on chromosomes with and without sister chromatid exchanges using acridine orange staining was registered, thus indicating BrdU incorporation in these regions of both CVS and ET.

Indeed, ET BrdU-antibody signals was observed in these heterochromatin regions as well as along chromosome arms. However in CVS BrdU-antibody signals were detected along of chromosome arms in both chromatids and asymmetric in Yqh region, which indicated that cells passed one cell cycle, but no any signals was detected in 1qh, 9qh and 16qh regions.

The reasons of this unusual cytogenetic phenomenon are discussed, such as technique artifact, cell cycle specificity or underreplication DNA constitutive heterochromatin of CVS.

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J03.08
A case of monosomy 9q: Clinical data and molecular cytogenetic characterization of a patient

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High resolution cytogenetics analysis techniques are becoming an indispensable tool in the investigation of idiophatic mental retardation and dysmorphic syndromes, allowing previously unrecognized chromosomal rearrangements to be identified.

In recent years many new syndromes have been established, including the clinically recognizable 9q subtelomeric deletion syndrome, in patients with severe mental retardation, hypotonia, brachycephaly, flat face with hypertelorism, synophrys, anteverted nares, cupid bow or tented upper lip, everted lower lip, prognathism, macroglossia, conotruncal heart defects and behavioral problems. The minimal critical region responsible for this 9q subtelomeric deletion (9q34) syndrome has been estimated to be 1 Mb and comprises the euchromatin histone methyl transferase 1 gene (EHMT1).

We describe a child with a *de novo* 9q microdeletion detected by subtelomeric FISH probe. This case contributes to the better characterization of the genotype/phenotype relationship of chromosome 9 rearrangements and emphasizes the role of molecular cytogenetics in the characterization of rare chromosomal disorders.

J03.09
Untargeted cytogenetic effects in human 25 years following Chernobyl accident

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25 years following Chernobyl accident one of the most actual problems of human radiation cytogenetics is evaluation of late untargeted cytogenetic effects, especially, different types of chromosome instability and bystander effect. Such effects in some critical groups of Chernobyl accident victims had been investigated using the following models: long-termed cultures of human blood lymphocytes; G_2-bleomycin sensitivity assay; mixed human lymphocytes culture consisted of cells differed by cytogenetic sex markers. In children born to irradiated parents increased frequency of chromatid breaks in long-term cultures confirmed expression of delayed chromosome instability in consequent mitosis; appearance of stable aberrations may testify about

transmissible chromosome instability. Among unexposed donors, liquidators and shelter's personnel no more than 33% persons hypersensitive to testing bleomycin exposure had been identified that can be considered as genetically caused phenomenon. Among patients recovered from acute radiation sickness ~58% persons expressed hidden chromosome instability that confirmed possibility of modification of inherited chromosome susceptibility to mutagens by high doses of ionizing radiation. Under joint incubation of targeted lymphocytes from Chernobyl male-liquidators with intact female lymphocytes the frequency of chromosome aberrations in bystander cells was significantly higher than their background level. The difference between spectrum of aberrations in exposed and intact cells had been established - in targeted cells cytogenetic markers of irradiation dominated just as in bystander cells chromatid breaks mainly induced. The data received confirmed reality of untargeted cytogenetic effects in delayed terms following Chernobyl accident that can be risk factor for realization of late medical consequences of human radiation exposure.

J03.10
Three cases of de novo isodicentric X chromosome

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Isodicentric X chromosomes, which formed by fusion of two X chromosomes, are rare. The phenotypic effects of this X chromosome aberration are variable and depend on the amount of material deleted and whether the chromosomes are fused by the short or the long arms. Despite the variable presence of a 45,X cell line, they form two distinct groups. Those joined by the short arms reveal shorter than average stature, while those attached by the long arms exhibit normal stature, both with gonadal dysgenesis. Other Turner's syndrome features can occasionally be present in both groups, but are more frequently associated with short arm fusions.

We report three cases which were referred to our Department due to primary amenorrhea. First case was 21-year-old, 159 cm height, 55 kg weight and with intermediate IQ. She presented with the features of Turner. Sonography report showed lack of ovaries and hypoplasia of uterus. Her FSH and LH were high. Karyotyping was performed on peripheral blood lymphocytes using banding techniques according to standard Methods: Her karyotype was: 46,X,idic(X)(q24).

Second case was 20-year-old, 156 cm, 63 kg and subnormal intelligence. She showed some Turner features. Her sonography revealed hypoplastic uterus and absence of gonads.She had elevated FSH and LH.

Her karyotype was: 45,X [80%] / 46,X,idic(X)(q22) [20%].

Third case was 23-year-old, 170 cm, 51 kg, with gonadal dysgenesis. She showed raised FSH and LH.

Her karyotype was: 45,X [8%] / 46,X,idic(X)(p22.3) [72%].

Chromosomal studies of the parents of all three cases and all their sibs revealed normal.

J03.11
Trisomy 21 in a boy whose mother is a carrier of a marker chromosome

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A marker chromosome (mar) is a structurally abnormal chromosome in which no part can be identified. The significance of a marker is very variable as it depends on what material is contained within the marker. It is essentially a partial trisomy.

Here we report a 8-month -old boy with history of open heart surgery because of his heart located in right side. He had developmental delay. His father had also same heart problem. Her mother was deaf and mute. She had irregular menstrual cycle and received treatment.

His parents were first cousin. Karyotyping after lymphocyte culture revealed 47,XY,+21, del (21)(q22.3) karyotype. The mother had 47,XX,+mar karyotrype. C-banding revealed that the marker had heterochromatin origin.

Here we discuss the risk of carrying a marker chromosome with no major clinical symptoms and trisomy of small chromosome in the next progeny

J03.12
Subtelomeric chromosomal rearrangements in idiopathic mental retardation by fluorescent in situ hybridization

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Mental retardation (MR) affects about 3% of the populations. Final diagnosis could be reached in approximately one third of all cases, and no diagnosis is available in the rest of the patients. Chromosomal abnormalities comprise 30-40% of moderate to severe mental retardation; and nearly 30% of mild mental retardation. Standard cytogenetic analysis is capable of detecting DNA rearrangements of larger than 3-5 Mb. Cryptic *subtelomeric* rearrangements are *responsible* in idiopathic *mental retardation* etiology for 5% to 10% of cases. *The aim of the* study is to evaluate the incidence of subtelomeric cryptic rearrangements in cases with idiopathic mental retardation. Eighty-six moderate to severe mentally retarded patients who were referred to the Department of Medical Genetics between 2008-2010 years. Definite perinatal brain injury, abnormal karyotype, positive FMR1 gene mutation, inherited metabolic or specific neurodegenerative disorders were excluded from the study. Fluorescent in situ hybridization analysis with
subtelomeric specific probes was used to detect cryptic rearrangement. Six cases were identified with subtelomeric rearrangements (7%). One had de novo deletion whereas five had unbalanced translocations due to balanced translocations of
the parents. There was no consanguinity between the parents in any of the cases and all had both mental retardation and multiple congenital anomalies.This study showed ones more that the subtelomeric rearrangements are one of the most significant cause of idiopathic MR with dysmorphic features.

J03.13
Report of a Down syndrome baby girl with double chromosome abnormalities of trisomy 21 and an isochromosome X

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Down syndrome features was referred to the medical genetics department of Sarem Hospital. Her parents had non consanguineous marriage with no family history of abnormal child. Mother was 21 years old at the time of the pregnancy and had no complication except oligohydramnios. Baby girl showed typical clinical features of Down syndrome including: hypotonia, hypothyroidism, sandel gap, short nose, clinodactyly, up ward slanted palpebral fissure, deep nasal bridge, short neck, small ear, high narrow palate and bilateral simian crease. She also had heart complication and jaundice from the birth time. Chromosomal analysis was carried out using standard high resolution GTG banding technique. The karyotype was abnormal with a double chromosome abnormalities containing a pure trisomy 21 and an isochromosome X described as 47,X,idic(X)(P11.2),+21. Parent's karyotypes were normal. The clinical profile of the Patient in relation to the double chromosome abnormalities will be presented.

J03.14
Cytogenetic studies among 50 Iranian patients with ambiguous genitalia

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Human sexual differentiation is a highly complex process that is under the control of multiple genes and hormones. Abnormalities in normal sexual differentiation are relatively common and occur one in 4500 live births, hence it needs immediate and rational management and assignment of sex for rearing should be guided by the etiology of the genital malformation. Using cytogenetic analysis may help determining suspicious cases, since ambiguity can result from chromosome abnormalities.

Diagnosis and sex determination were made using a combination of clinical, radiological and laboratory investigations.

Chromosomal studies were performed on peripheral blood samples using conventional GTG banding techniques in more than 100 metaphases for each sample.

50 patients with ambiguous genitalia were referred for confirmation of sex by karyotyping. They were aged between one month to 24 years.

Our results showed that 29 patients (58%) had normal karyotype.

The karyotypes of the rest of the cases were as follows:

14 patients (28%) were congenital adrenal hyperplasia (CAH), twelve of whom showed 46,XX and two cases were 46,XY karyotype (both 3B-hydroxylase deficiency).

4 patients (8%) were 21-OH deficiency.

1 patient (1%) was phenotypically male but 46,XX karyotype (XX male).

2 cases (2%) have 46, XX/46,XY karyotype. There is a great discrepancy between phenotype-karyotype in individuals having a chromosomal mosaicism.

J03.15
Cryptic imbalances detected by array CGH in cases with identified cytogenetic alterations

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Array-CGH has been assuming a major role in the diagnosis of patients with mental retardation, developmental delay and multiple congenital abnormalities and in the further characterization of cytogenetically detected alterations. Among the available platforms, BAC and oligonucleotides, the last ones allow the detection of smaller imbalances, offering a higher diagnostic yield. Meanwhile, a drawback of this higher resolution is that the generation of more data makes the interpretation increasingly challenging. This is particularly important when talking about copy number variants (CNVs), due to the progressive discovery of CNVs of unclear significance.

We performed array-CGH using Agilent 180K, an oligonucleotide microarray with 180 000 sixty-mer probes and 17 Kb average probe spacing. The patient's samples were hybridized in a loop strategy, where the three samples are labeled in the two colour dyes, Cy3 and Cy5, and then each patient sample is hybridized against the two other samples.

In a small cohort we have identified a cytogenetically detected chromosome 21 duplication that turned out to be an insertion from chromosome 4, a 6q21 deletion in a patient with a t(13;15) and a cryptic 20p12 deletion in Alagille syndrome region in a del2p patient.

Our experimental group shows that more than one structural chromosomal alteration in the same patient might occur and that its frequency is probably underestimated. Array-CGH will allow the

identification of cryptic imbalances that otherwise would be missed.

J03.16
Couples with two or more spontaneous abortions during the years 2000-2010 - performed in Center for medical genetics and immunology- Montenegro

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Research has shown that couples with balanced translocations are more likely to have miscarriages and increase risk for aberration offspring than couples without balanced translocations. The reported incidence of balanced chromosomal translocations in couples with multiple spontaneous abortions (SABs), for years 2000- 2010 performed in Centre for Medical Genetics and Immunology KCCG - Montenegro. After genetic counselling, family pedigree was drawn by genetic counselor. Some couples had more spontaneous abortions plus a malformed child or stillbirth. Cytogenetic analysis on blood specimens was needed for 325 couples (650 cases) and karyotyping was conducted by analysis of G-band.Chromosomal status was analysed using CytoVision,4.02 Applied Imaging. Balanced translocations were found in 25 cases with an incidence of 3,85%, indeed 7,69% couples. Of the 25 persons with balanced translocations 4 persons had Robertsonian translocation, another had translocation among different autosomes chromosomes.

J03.17
A mosaic Turner girl with ring chromosome X

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Women with the ring X chromosome are more likely to have psychological sequelae but are less likely to have structural congenital abnormalities, and spontaneous menses occur in about a third.
A 12-year-old girl was referred to our laboratory for chromosome study because of short stature (about 1 meter). Her IQ was within normal range. Other than being short she was physically and mentally normal. Her mother had to consume drug during the pregnancy since she had kidney disease. The karyotype of the patients was found to be 45,X[50]/46,X,r(X)(p?q?)[4].
The clinical manifestation of this patients will be discussed and compared with the similar reported cases.

J03.18
Screening of mitochondrial DNA mutations in the tRNAGlu gene and in tRNA Leu in buccal mucosa and in leucocytes in a Tunisian family with mitochondrial diabetes

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Diabetes mellitus (DM) is a heterogeneous disorder characterized by the presence of chronic hyperglycemia. Genetic factors play an important role in the development of this disorder, and several studies reported mutations in nuclear genes implicated in the insulin function. Besides, DM can be maternally transmitted in some families, possibly due to the maternal mitochondrial inheritance.
In this report, we screened a Tunisian family with mitochondrial diabetes for the m.3243A>G and the m.14709T>C mutations, respectively, in the tRNALeu(UUR) and the tRNAGlu genes.
The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and the sequencing analysis in the leucocytes and the buccal mucosa in the members of this family showed the absence of the m.3243A>G mutation and the presence of the heteroplasmic m.14709T>C mutation in the tRNAGlu gene in the two tested tissues.
We conclude that the m.14709T>C mutation in the tRNAGlu gene could be a cause of mitochondrial diabetes in Tunisian affected family. In addition, the heteroplasmic loads of the m.14709T>C mutation in the leucocytes and buccal mucosa didn't correlate with the severity and the onset of mitochondrial diabetes in the tested Tunisian family suggesting the presence of environmental factors or nuclear modifier genes. Moreover, our results showed that the clinical manifestations of mitochondrial diabetes in this tunisian family appear only in females. These results suggested the presence of a nuclear modifier factor specific to females which enhances the apparition of diabetes.

J03.19
The role of Streptococcus pyogenes and some metabolites in formation of genomic instability by tonsillitis of patient.

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We investigated content of two metabolites: malonic dialdehid (characterized the intensity of lipid peroxidation in cell membrane) and intercellular DNA (showed the motion of DNA from the cell).
We used extract of Streptococcus pyogenes as primary initiator increasing the intensity of endomutagenesis. It was inspect ed 48 patients with tonsillitis - 38 patients with genomic instabilities and 8 patients with low level of genomic damaging. The concentration of metabolites in blood plasma was determined on third day after beginning of disease.
Mutagenic activities of Streptococcus pyogenes extract was carried out by abdominal injection to white mice and processing of seeds of Crepis capillaries.
It was established that content of malonic dialdehid and intercellular DNA in blood plasma of patients with genomic instabilities exceeded the normal concentrations of ones in 3 times and more. In patients without genomic instabilities the content of above-mentioned metabolites was approximately normal. We worked up the method for determination of free iDNA and iDNA binding with cell membrane. It gives possibility to determine the intensity of apoptosis and necrosis of cells in patients with tonsillitis.
It was also established that the number of micronuclei in erythrocytes of peripheral blood of mice was increased after injection of Streptococcus pyogenes extract in comparison with the mice without injection.
Number of chromosomal aberration in seeds of Crepis capillaries after exposition with extract of Streptococcus pyogenes was significantly riser in comparison with control samples. Mutagenicity of extract depends from the bacterium concentration and rises with increasing of concentration.

J03.20
Cytogenetic investigation among women with multiple recurrent abotiotions

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Some of the cases with multiple recurrent abortions are caused by chromosomal aberration in one of the parents. Many investigations have been carried out in various countries to determine the role of chromosomal aberrations in couples with repeated fetal loss. Approximately 15 to 20 % of pregnancies in which the fetuses are affected with chromosomal abnormalities end to spontaneous abortion. A proportion of spontaneous abortions is caused by a balanced chromosomal aberration in one of the parents, which is of importance in genetic counseling. Several studies have been done among couples with multiple recurrent abortions.
This study was carried out among Iranian couples with the same problem. A total of 944 cases (472 couples) with a history of multiple recurrent abortions were examined, and chromosomal analysis was done using the G-banding technique.
26 females (5.5%) and 21 males (4.4%) were found to have abnormal karyotypes. These abnormalities included: one case with a mosaic karyotype 46,XX/46,XX,t(21/21),+21, 8 balanced reciprocal translocations, 14 Robertsonian translocations, 13 inversions, 4 marker chromosomes, 2 cases with fra(16)(q21), and four cases of X chromosome mosaics.

P04 Reproductive genetics

P04.01
Preventive examination of patients with balanced translocation

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Karyotypes of 5416 patients were examined at Dept. of Medical Genetics between January 2007 and December 2010. 25 men and 38 women had balanced translocation in their karyotype. Families of these patients were offered genetic consultancy with cytogenetic examination, its purpose was to find out whether translocation is inherited or arisen de novo. In cases when couples planed a child, prenatal genetic diagnostic eventually preimplantation genetic diagnosis was offered too. Free of charge analysis of frequencies of chromosomally unbalanced sperms in ejaculate was offered to adult men (17) but only 4 (23 %) accepted this offer. The frequencies of unbalanced sperm ranged from 5.8 to 23.5 % in Robertsonian and from 40.1 to 70.3 % in reciprocal translocation carriers. It is noteworthy that if a translocation (balanced or unbalanced) was found in children patient then in 80 % cases parents and other relatives underwent cytogenetic examination. When translocation was found in adult (usually examined because of infertility), in only 20 % cases their relatives underwent the examination.

P04.02
Functional analysis of CCDC54, a protein likely involved in spermatogenesis.

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In search for proteins involved in the complex process of spermatogenesis, coiled-coil domain containing 54 protein (CCDC54), a human testis-expressed protein with unknown function, was studied. Expression of this gene is predomintantly in testis and in silico analysis of the CCDC54 gene suggested it is a conserved gene. A yeast two hybrid screening was performed, which identified Testis specific Zinc Finger Protein (TZFP), a transcriptional repressor, as a binding partner of CCDC54. To further investigate the effect of the binding of CCDC54 on the activity of TZFP, a luciferase reporter assay was performed. This suggested that CCDC54 is a negative co-regulator of TZFP. Immunohistochemical staining of human testis tissue shows an overall expression of the CCDC54 protein in the seminiferous tubules.
At the DNA level, conservation of the CCDC54 gene was verified and mutation analysis was performed. To detect the reported c.316G>T or p.Glu106X polymorphism, a restriction digestion with BccI was performed on a control group of 150 European normozoospermic men, but the polymorphism was not found. The CCDC54 gene of 16 European men with a maturation arrest of spermatogenesis and 32 European men with Sertoli cell-only syndrome (SCOS) was sequenced and only one missense mutation was found in 2 SCOS patients.
These preliminary results suggest that CCDC54 could be an important protein for spermatogenesis.

P04.03
Early embryonic developmental arrest and chromosomal aneuploidies

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OBJECTIVE: Despite strongly improved culture conditions approximately 10 - 20 % of IVF embryos show a permanent cell cycle arrest state. Of special interest for this study is an increased rate of numerical chromosomal abnormalities found in embryos with early developmental arrest.
MATERIALS/METHODS: The study was approved by the local ethical review board and can be accomplished in accordance with the strict Austrian legal regulations. Arrested embryos used in this study were donated by patients undergoing IVF treatment for infertility, and written patient consent was obtained in each case. Embryos were considered to be arrested when no cleavage had occurred during 48 h. Blastomeres of embryos with developmental arrest were analysed using Fluorescence *in situ* hybridisation (FISH). Arrested embryos were classified into different categories: normal diploid (euploid), homogeneously abnormal (aneuploid), abnormal mosaics, chaotic (complex), polyploid and haploid.
RESULTS: The most frequent finding in the study was the chaotic pattern with various numerical chromosome abnormalities, which can be different from cell to cell. Polyploidy comes a close second and represent a special case, since here probably not the polyploidy is the reason for embryonic arrest, but the arrest is the cause for the polyploidy.
CONCLUSION: Almost 70% of the analysed arrested embryos displayed chromosomal abnormalities, suggesting that the elevated levels of chromosomal abnormalities represent a not negligible factor for developmental arrest.

P04.04
Genomic alterations in Endometriosis

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The origin and genetic background of endometriosis are still unknown. Several studies have reported presence or absence of genomic alterations and copy neutral loss of heterozygosity (LOH) in eutopic and ectopic endometrium of women with endometriosis. In this study, we used high-density SNP-arrays to determine inherited DNA copy number variations (CNVs), *de novo* somatic copy number aberrations (CNAs) and LOH pattern in DNA samples extracted from endometriosis lesions and compared to the original situation in blood cells and eutopic endometrium to reveal the genetic component of endometriosis development. Peripheral blood samples, endometrial biopsy and laser microdissected ectopic endometriotic tissues were collected from 11 women with endometriosis. The data was analysed using QuantiSNP and PennCNV programs. We examined the genomic changes in microdissected endometriotic foci matching with endometrium and blood samples. An average of 9 CNVs was detected per investigated genome with a size range from 700 bp to 350 kb. We found that CNVs and LOH pattern are largely invariable in different compartments (blood, endometrium and ectopic tissue) of each individual patient. Likewise, no endometriosis-specific CNVs or LOH regions were found when different endometriosis patients were compared. Majority of detected CNVs were common CNVs. Also, ectopic endometrial tissue showed no large chromosomal aberrations in our study group. Therefore, we conclude that there is no convincing evidence that endometriosis originates from genomic alteration in eutopic endometrium and the disease is not accompanied by extensive genomic rearrangements.

P04.05
Search for genes associated with number of pregnancies

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While data about the genetic background of different infertility-related conditions in women is growing rapidly, the genetic component of general fertility and fertility potential is still largely unknown. Research in this field is hindered by the fact that in modern societies the number of pregnancies is mostly determined by socioeconomic, psychological and environmental factors, and the availability and awareness of contraception, whereas the original genetic determinants fade into the background. In Estonia modern contraceptives became available in the beginning of the nineties, illustrated by the fact that in 1994 only 234 out of every 1000 fertile women were using effective means of contraception and the abortion rate was 54 per 1000 women of reproductive age.

We analyzed data from 944 women aged 40-101 (average age 65.2 years) with an average of 3.5 pregnancies (range 1-14); furthermore, 43% of the studied women had had at least 4 pregnancies. Preliminary analysis of data obtained from genome-wide association study revealed possible association between the number of pregnancies and regions in or near genes previously associated with menarche, pathophysiology of placental diseases, steroid hormone receptor modification, maintenance of normal pregnancy, VEGF signalling and adiponectin levels.

In conclusion, although research regarding the genetic component of fertility potential is complicated due to numerous confounding factors, our results suggest genetic determinants can still be found. Analysis of data obtained from further studies might reveal additional associations and confirm current findings.

P04.06

Chromosomes aneuploidies detected in decondensed sperm nuclei using FISH

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The aim of this study was to evaluate the hypothesis that infertile men, having oligoasthenozoospermia present a higher grade of chromosomal aneuploidies than men with normal fertility. FISH technique allows the study of a significant number of spermatozoa in a relatively short period. This is very important for the correct evaluation of the patients with oligoasthenozoospermia, knowing that the frequency of chromosomal anomalies in spermatozoa of infertile men is 10 times bigger than in men with normal fertility. In this study we included 15 men with oligoasthenozoospermia and a control lot of donors for whom at least 1000 spermatozoa were evaluated. Sperm count revealed a number varing betwen $3x10^3$ and $3x10^6$ and 50-80% of the spermatozoa were imobile. All the patients inluded in this study had normal constitutional karyotype. We performed FISH technique using Abbott Vysis 13, 18, 21, X and Y chromosomes probes following the standard protocol provided by the manufacturer with slight modifications. We assessed the chromosomes that have most frecquently major clinically signifiance in children with aneuploidies. The aneuploidies rate of the studied chromosomes in our oligoasthenozoospermic patients spermatozoa was 13.61%, the higher incidence was recorded for gonosomes followed by disomy of chromosomes 13, 21, 18. Our results show an increase frequency of sperm chromosome abnormalities in infertile patients compared with control donors. Patients with normal karyotype, low sperm concentration and reduced spermatozoa motility have an increased risk of producing spermatozoa with chromosomal number aberrations, particularly for the sex chromosomes.

P04.07

Intermediate and normal sized CGG repeat on the FMR1 (fragile X) gene does not affect ovarian response

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INTRODUCTION: Fragile X syndrome is not just the most common cause of inherited mental retardation, but of of low ovarian response and premature ovarian failure (POF) as well. Gleicher et al reported a direct statistical association between the number of CGG repeats (at 35 to 55) an ovarian reserve.

The goal of this study is investigated whether CGG repeats on the FMR1 gene have predictive value for ovarian response to stimulation with gonadotropins and cycle outcome during an oocyte donors programme.

MATERIALS AND METHODS: We performed fragile X genetic screening routinely to all our oocyte donors from 2008. We include the results of X fragile genetic screening from 204 (141 control and 63 study groups) oocyte donors. The study population was divided into three groups: women with 35-39 repeats (n=34), 40-45 (n=12) and >45 (n=17).

RESULTS: No significance differences in cycle outcome was observed between groups, neither between different subgroups. According to ovarian stimulation, no differences were observed in donor age and number of oocyte yield between control and population groups and subgroups. Gonadotropin dosages and days of stimulation correlated with respect to the number of CGG repeats. These results disagreed with previously work performed on infertility patients.

DISCUSSION: For the first time we present data showing the relation between normal and intermediated sized CGG repeats on FMR1 gene and ovarian stimulation using a non-confusion model as oocyte donor. Our data suggest that ovarian stimulation does not be affected by CGG repeats on normal and intermediated range.

P04.08

The utility of methylation sensitive high resolution melting assay at imprinted genes in the diagnosis of complete and partial hydatidiform mole

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Background: Hydatidiform mole (HM), represents a disorder of genomic imprinting, a phenomenon whereby genes are monoallelically expressed from the maternally or paternally-derived copy of the gene. HM are classified as partial (PHM) or complete (CHM). The aim of this study was to analyze and to compare the methylation patterns of imprinted genes in CHM and PHM.

Material and Methods: We used methylation-sensitive high-resolution melting technique (MS-HRM) for the methylation analysis of three paternally expressed genes, *SNRPN*, *KCNQ1OT1* and *GNAS-XLaS*, that are normally only methylated on the maternal allele, and a maternally expressed gene, *H19*, which is normally only methylated on the paternal allele. DNA samples including 25 CHM and 20 PHM were scored as hypermethylated, normal, or hypomethylated.

Results: We found loss of cytosine methylation in 19 CHM and 1 PHM at *SNRPN* DMRs, 20 CHM and 1 PHM at *KCNQ1OT1* DMRs, and in 24 CHM and 3 PHM at *GNAS-XlaS* DMRs. Hypermethylation at *H19* DMRs was showed in 21 CHM and 1 PHM. Abnormal DNA methylation patterns at all imprinted genes were highly correlated to CHM than PHM ($p<0.05$).

Conclusion: Our data confirm that abnormal methylation status of imprinted genes is significantly associated to androgenic CHM characterized by diffuse trophoblastic hyperplasia and the lack of foetal development. So, we confirm the correlation between phenotypic variability of different forms of HM and various degrees of disorder of imprinting. MS-HRM analysis is a rapid, specific and sensitive method, which can be applied for the accurate differentiation between CHM and PHM.

P04.09***

Prenatal diagnosis for Huntington disease in the Netherlands (1998-2008)

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Prenatal diagnosis for Huntington's disease (HD) is possible using linkage analysis since 1987 and using direct mutation analysis since 1993, when the HTT-gene was identified.

In a previous study in the Netherlands (Maat-Kievit et al. 1999), prenatal diagnosis for HD was categorized for the period 1987-1997. In this study we categorized the next decade: 1998-2008.

We studied the medical records from all couples that had prenatal diagnosis between 1 Jan 1998 and 31 Dec 2008, to collect data regarding reproductive history, DNA results (predictive and prenatal) and decisions made in pregnancies.

In the study period 126 couples had 218 prenatal tests, 187 (86%) direct mutation analyses and 31 (14%) exclusion tests. In the previous decade, figures/data were respectively 41 and 31.

Of the couples that had their first prenatal test in this period, 13% opted for an exclusion test.

We identified a new category of at risk parents opting for prenatal testing: carriers of a CAG repeat in the low penetrance or intermediate range. In 92 prenatal tests the result indicated an affected fetus. Of those, 11 pregnancies were continued (12%).

Though the proportion of direct tests has increased, linkage analysis is still the first choice for a minority. We found that quite some pregnancies with an unfavorable result were continued. The circumstances that led to this decision will be specified.In a separate survey we will investigate how many couples switched to preimplantation genetic diagnosis at some point in their reproductive choice.

P04.10

Idiopathic Hypogonadotropic Hypogonadism in Finland

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Idiopathic hypogonadotropic hypogonadism (IHH) is a clinically and genetically heterogeneous disorder characterized by absent or incomplete puberty with anosmia (Kallmann Syndrome; KS), or with a normal sense of smell (normosmic IHH; nIHH). The molecular genetic cause can only be identified in ~30% of patients. We investigated epidemiological, clinical, and genetic features of IHH in Finland. The minimal incidence estimate of KS was 1:48 000, with a clear difference between males (1:30 000) and females (1:125 000) (p=0.02). The phenotypes of 30 KS probands (25 men; 5 women) ranged from severe IHH to partial puberty. Mutation analysis of *KAL1*, *FGFR1*, *FGF8*, *PROK2*, *PROKR2*, *NELF*, *CHD7*, and *WDR11* genes revealed mutations in *KAL1* (3 men) and *FGFR1* (all 5 women vs. 4/25 men), but not in other genes. In addition, 15 patients with nIHH were phenotyped, and screened for mutations in *FGFR1*, *FGF8*, *PROK2*, *PROKR2*, *NELF*, *CHD7*, *WDR11*, *GNRHR*, *GNRH1*, *KISS1R*, *KISS1*, *TAC3*, *TACR3*, *FSHB* (6 females), and *LHB* (9 males). Three patients were compound heterozygotes for *GNRHR* mutations, but no mutations were found in other genes. The lack of mutations in *PROK2*, *PROKR2*, *TAC3*, and *TACR3*, implicated in recessive IHH, may reflect the special genetic features of the Finnish population. No evidence of oligogenic inheritance, suggested to contribute to IHH, was observed. Altogether 30 patients (67%) remained without identified mutations in any of the known IHH genes, and we expect to identify mutations in previously undescribed genes underlying IHH among the Finnish patients.

P04.11

Sperm and lymphocyte low level aneuploidy in patients with olygozoospermia

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Karyotype abnormalities contribute to male infertility. But normal male karyotyte does not exclude low level aneuploidy in somatic cells and sperm aneuploidy. Abnormal semen parameters in chromosomally normal men could be a risk factor of sperm aneuploidy. Numerous data are available regarding sperm aneuploidy in patients with oligozoospermia (OS) but they are very disconnected, and there are no acceptable normative parameters.

We investigated germ and somatic cells of 20 men with normal male karyotype: 10 patients with oligozoospermia and 10 fertile normozoospermic men. The karyotyping was performed by GTG-banding technique. Aneuploidy level for chromosomes 13, 18, 21, X, and Y was investigated in cultivated lymphocytes and fixed decondensed sperms by FISH. Limits and confidence intervals were scored for control group. Semen analysis was performed according to the standard WHO criteria.

A total of 20000 sperm and 20000 lymphocytes were scored for five chromosomes. We found significant differences in aneuploidy level for five chromosomes between OS patients and control group both in lymphocytes: 2.04±0.21% and 1.04±0.07% (increase of limit) and in sperm: 2.17±0.28% and 1.17±0.14% (P=0.001). Inverse correlation was found between sperm aneuploidy level and sperm concentration in ejaculate (r=0.644). There was a mild association between aneuploidy level in blood and germ cells for chromosomes 13 and 18 (r=0.426 and r=0.392).

In our investigation we determined limits of sperm and lymphocyte aneuploidy in the control group. These data can be useful for cases of mosaicism (especially low level) and sperm aneuploidy analysis. OS patients had elevated aneuploidy level.

P04.12

Hemochromatosis gene mutations (HFE) in infertility

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Hemochromatosis - an autosomal recessive disorder, the most common causes of it are two mutations of HFE gene: C282Y and H63D. The HFE gene mutations lead to increased iron absorption in the gastrointestinal tract. Detailed clinical symptoms occur in 4-5 decade of life. Before that, the iron is gradually accumulates in the body tissues. So violations of the male reproductive function as impotence, testicular atrophy, azoospermia, and so female reproductive function as amenorrhea may occur long before the major symptoms of hemochromatosis. Based on these data and high-frequency carrier of fhe disease in different populations, the search of C282Y and H63D mutations of HFE gene was carried in a group of 132 men with infertility diagnose. As a control, we studied a sample of 204 not clinically surveyed residents of the Russian Federation.

C282Y mutation in the heterozygous state was detected in 12 patients from the infertile group and in 13 ones from the group of population controls, H63D in 47 and 35 ones, respectively. H63D mutation in the homozygous state was detected in 2 patients from the infertile group and in 7 persons of the control group, 1 patient and 2 persons from the comparison group were compounds for investigated mutations. Neither statistically significant differences of C282Y and H63D mutations allele frequencies for studied groups no differences between the incidence of two mutations carriers were found.

Thus, it was found that the HFE gene mutations do not contribute to the development of infertility among residents of the Russian Federation.

P04.13

Study of GT-repeat expansion in Heme oxygenase-1 gene promoter as genetic cause of male infertility

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A reduction in the level of HO-1 protein has been observed in the seminal plasma and reported to be associated with oligospermia and azoospermia in men with infertility. The length of GT-repeats polymorphic region in the promoter of human Heme oxygenase-1 gene (HO-1) alters the level of its transcriptional activity in response to oxidative stress. This is the first study to investigate the association between GT-repeat expansion in the promoter of HO-1 and male infertility in Iranian population. The GT-repeats alleles were determined in 100 cases and 100 normal controls by molecular analysis method such as PCR-PAGE, ABI fragment analysis genotyping and sequencing. Two classes of S allele with 27 GT-repeats were specified as 4 to 6 alleles. The L allele frequency was significantly higher (54.5%) among case group than that in the normal controls (37.5%). Statistical analysis provide significant relationship between L alleles and male infertility

(P<0.001). This study shows for the first time that GT repeat expansion in promoter of the HO-1 gene is associated with oligospermia and azoospermia in male infertility among Iranian infertile cases.

P04.14
Cytogenetic investigations in Assisted Reproductive Technology (ART) - experiences of Center of Reproductive Medicine (Belarus)

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Cytogenetic investigations of sterile patients, couples with reproductive failures, fetuses suspected for chromosomal disorders play an important role in diagnostics of etiology of infertility, fetal loss, handicapped offspring. We present a scheme of genetic service, cytogenetic results, selective fetal reduction data. Genetic service of CRM provides counseling of infertile couples, pregnant women after ART with fetal loss or abnormalities; cytogenetic and molecular analyses, prenatal combined (US + serum markers) screening, genetic risk calculation. Among 3865 examined patients (lymphocytes, GTG) aberrations were detected in 1.2%. Balanced rearrangements (71%) presented Robertsonian and reciprocal translocations, inversions of chromosomes 2;5;6;7;9;10;X;Y. Unbalanced aberrations (29%) included numerical gonosome abnormalities, markers (47,XYY; 47,XYY/46,XY; 47,XY,+mar; 47,XY,+mar/46,XY; 45,X/46,XY,gh+; 47,ÕÕÕ; 47,XXX/46,XX; 45,X/46,XX; 45,X/48,XXXX/46,XX), X and Y deletions. 1312 pregnant women underwent screening in the 1st trimester. Couples with increased risk - advanced maternal age (27.7%), calculated risk for combined screening ≤1:360 (9.7%), parental balanced rearrangements - were selected for prenatal karyotyping (CVS, GTG). 14 reductions of fetuses with aneuploidy were performed in the 1st trimester using Berkowitz's method (one test-positive couple refused which resulted in the birth of a child with Down's syndrome). 13/14 terminated cases presented twin pregnancy after IVF (4) or IVF+ICSI (9) with one affected fetus (trisomy 21, 18, 13, monosomy X; inherited structural autosomal unbalance). Pregnancies resulted in deliveries of healthy newborns. Conclusion: Prenatal testing for fetal abnormalities is a necessary preventive measure - in cases of elevated risk of severe consequences for an offspring's health, reduction of the affected fetus is preferred.

P04.15
Diagnostic testing for Male Factor Infertility using APEX microarrays

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Objective: Male factor infertility (MFI) issues are causative in approximately 15-20% of infertile couples. The unfavourable genetic background is thought to cause for 15-30% of male factor infertility cases. Therefore, the correct determination of genetic basis of infertility is very important for further treatment of patients. Here, we demonstrate a single-step Arrayed Primer Extension (APEX) based microarray assay for the MFI diagnosis.

Design & Method: The MFI-APEX assay enables analysis of numerous genetic factors simultaneously, like AZF-microdeletions; Klinefelter syndrome; mutations in cystic fibrosis transmembrane conductance regulator (CFTR) gene; genes involved in folate metabolism; cryptorchidism, hypogonadism, spermatogenic failure linked genes; and polymorphisms in androgen receptor gene. For validation of the MFI-APEX assay we used blood samples obtained from 22 infertile men and 10 fertile men as controls.

Results: We developed MFI-APEX microarray assay for the detection of genetic factor of the male infertility. Analysis of the APEX results revealed that 14 out of 22 patients had Klinefelter syndrome; 2 patients had Klinefelter syndrome and mutations in CFTR gene; 2 patients had AZFbc microdeletion; 5 patients had AZFc microdeletion; and 1 patient had AZFc deletion and mutation in CFTR gene. No alterations were found in fertile men with MFI-APEX assay.

Conclusions: Our experiments demonstrated that MFI-APEX assay is suitable for the rapid single-step, robust, reliable and cost-effective detection of possible genetic cause for male infertility problem. Furthermore, MFI-APEX testing is recommended before the couple undergoes assisted reproduction in order to prevent the possible inheritance of the genetic lesion to the next generation.

P04.16
Array-CGH analysis in patients with maturation arrest of spermatogenesis

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Introduction. The past decades, many studies have been performed aiming to identify genetic causes of maturation arrest of spermatogenesis (MA). However, these studies were rather disappointing. In the present study, we looked for the presence of copy number variations (CNVs) in patients with MA and controls with normal sperm parameters

Material and methods. CNVs were identified using 244K arrays in 9 patients. For regions of possible interest, more patients and especially larger groups of control men were investigated by qPCR.

Results. In the patient and control groups, on average 25±4 and 24±7 CNVs were detected, respectively. After elimination of regions that are not containing any known genes and regions that were also detected as CNVs in controls, 32 regions remained. A further reduction to nine regions was done by eliminating deletions that are completely intronic (fi in PRKG), regions that were recently amplified in evolution (f.i. BAGE and PNMA genes) and regions with a well-known function not involved in spermatogenesis. The remaining nine regions are being investigated by qPCR in order to investigate large numbers of controls (>100) and to conclude whether the observed CNVs are common or related to the fertility problems. Preliminary results show that at least some of the regions are exclusively detected in the patients.

Conclusions. In this study, a small group of patients with MA have been thoroughly investigated using array-CGH. No common region was detected in the patient group that was absent in control samples. Six promising regions will be further investigated.

P04.17
Association of polymorphisms in eight genes (FASLG, JMJD1A, LOC203413, TEX15, BRDT, OR2W3, INSR and TAS2R38) with male infertility

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The analysis of polymorphisms in genes involved in spermatogenesis represents an exciting area of research in genetics of male infertility. Polymorphisms in these genes are considered potential risk factors which may contribute to the severity of spermatogenic failure. Here we have investigated the possible association of nine single nucleotide polymorphisms (SNPs) in eight different genes (FASLG, JMJD1A, LOC203413, TEX15, BRDT, OR2W3, INSR and TAS2R38) with male infertility. The SNPs were selected because they were found to be associated with azoospermia and /or oligozoospermia in a recent study (Aston KI et al, Hum Reprod, 25:1383-97, 2010).

We have analysed a total of 136 men with idiopathic infertility (60 azoospermic and 76 oligozospermic) and 161 fertile controls. Ninety three of the infertile men were Macedonians, 32 were Albanians and 11 men were of other ethnic origin. The control group was composed of 125 Macedonian and 36 Albanian men. The methodology included multiplex PCR/SNaPshot method, followed by capillary electrophoresis on ABI3130 Genetic Analyzer.

Of the nine SNPs evaluated, we have found significant association ($p<0.05$) for three. The most significant association was found for rs5911500, an intergenic SNP located on the X chromosome. This SNP as well as rs3088232 in BRDT gene showed association with azoospermia both among Macedonians and Albanians, while

rs11204546 in OR2W3 gene was also associated with azoospermia, but only among Macedonian men. Another SNP (rs34605051 in JMJD1A gene) was present with higher frequency among all groups of infertile men in comparison to fertile controls, but the differences were not significant.

P04.18
Analysis of *DLK1* gene and microRNA expression in male infertility due to idiopathic testicular dysfunction

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Cellular interactions between germ-line and somatic components of the testicular seminiferous tubule form a complex network, and are essential for efficient germ-cell development. Gene expression of Leydig cells is crucial to maintain male fertility.

We have assessed the expression profile of *DLK1* gene (preferentially expressed by Leydig cells) and its regulation by miRNAs (involved in posttranscriptional gene silencing) in impaired spermatogenesis.

We have analysed *DLK1* and three *DLK1*-target miRNAs *(miR-33a, miR-514* and *miR-377)* expression levels in testicular biopsies of 33 non-obstructive infertile men (patients), who showed total absence of germ cells (SCO; n=14), complete meiotic arrest (MA; n=7) and hypospermatogenesis (HS; n=12), and 16 men with obstructive azoospermia and conserved spermatogenesis (controls). The current method was the real time RT-PCR (7300 Real Time PCR System and TaqMan Assays) and the 2-ΔΔCt strategy. *PGK1* gene was selected as normalizer for *DLK1* data and *RNU6B* for miRNA data. The Mann-Whitney U test was used to analyse expression differences between groups.

Preliminary results show significant *DLK1* over-expression in the three patient subphenotypes compared to controls. The expression levels of the three miRNAs were found significantly increased in the SCO subphenotype.

These results suggest that changes in the expression of *DLK1* in Leydig cells might be associated to spermatogenic impairment. These differences in expression are not associated to *miR-33a, miR-514* and *miR-377* miRNA regulation. These data would contribute to a better understanding of the molecular mechanisms underlying male infertility.

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P04.19
Role of genetic factors and oxidative stress in male infertility

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Introduction: Infertility affects 15% of couples in their reproductive age group where 30 to 40% of males are the sole contributor and majority of these are idiopathic. Methods: The study included 200 men with idiopathic infertility and 100 fertile controls. The work involved cytogenetic and Y chromosome micro-deletion analysis, evaluation of seminal reactive oxygen species levels by chemiluminescence assay and sperm DNA damage by sperm chromatin structure assay (SCSA). Results and Discussion: Out of 200 infertile men screened, 13% harbored cytogenetic abnormalities. Azoospermia factor (AZF) microdeletions were detected in 9.5% infertile men, where AZFc deletion were predominant followed by AZFb and AZFa deletions. This frequency is similar to that reported in French & Danish population. Level of ROS in both washed and neat semen was found to be significantly (P<0.0001) higher in infertile men compared to controls. The average mean DNA fragmentation index (DFI) in infertile men was found to be 40.85% which was significantly higher (p<0.0001) compared to average mean DFI (25.62%) in controls. Conclusion: Cytogenetic abnormalities and Yq microdeletions are present in about 22% of men with idiopathic infertility. Analysis of sperm DFI and the cut off level of 30.42% is useful to predict outcome of ART as conventional semen analysis is a moderate predictor of fertility potential. Early detection of OS and suitable antioxidants therapy may prevent further deterioration of semen quality and improves chances of fertilization as it improves membrane integrity. Thus genetic analysis, assessment of ROS levels & DFI has both diagnostic and prognostic capabilities.

P04.20
Study of T886C SNP in TAS2R38 gene association with azoospermia and oligospermia in idiopathic infertile men.

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Back ground- Genome wide SNP association study (GWAS) by microarray method could identified several SNPs that had potential relevance to oligospermia and azoospermia have occurred due to idiopathic infertile men. One such study by Aston et al in 2010 has reported significant SNPs in European population. The most significant association found for an intergenic SNP (T886C) in TAS2R38 gene with male infertility. This SNP results in a conservative amino acide change of Isolucine to Valin.

Objective- This is the first study to evaluated association between T886C SNP in TAS2R38 gene with oligospermia or azoospermia in idiopathic infertile men in Iranian population.

Methods- This relationship has been studied in 96 idiopathic infertile men with azoospermia and oligospermia and 100 normal control men. Primer pairs were designed to amplify 192bp fragment contained T886C polymorphism. Analysis of SNP was performed by PCR-RFLP and High Resolution Melt (real time PCR-HRM corbett). Amplified fragment was sequenced to determine the PCR product and allelic identify.

Results- Genotype frequencies of the TT, TC and CC were as 20.8%, 34.4% and 44.8%, in the case group and 21%, 34% and 45% in the control group respectively. The prevalence of tested SNP was approximately similar in both infertile patients and fertile control groups. Statistical analysis showed no significant relationship P=0.9 for this SNP (T886C) in TAS2R38 gene.

Conclusion- This result is indicated that (T886C) in TAS2R38 gene was not associated with oligospermia and azoospermia that could caused idiopatic male infertility in Iranian population.

P04.21
Gene expression alteration of the YBX2 and DAZ genes is associated with male factor infertility

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Y chromosome microdeletions are the major molecular determinants for male infertility by removing key-genes for male germ cell development and maintenance. Despite the large number of studies about the molecular basis of male infertility the molecular mechanism leading to spermatogenesis disruption is still largely unknown. Several forms of male infertility can be determined by a common pathogenic mechanism related to alterations in testicular mRNA storage. DAZ and YBX2 are the main genes involved in the regulation of the stability and storage of male germ cell mRNAs. We previously analyzed using quantitative real-time SYBR Green PCR assays the YBX2 and its direct interacting genes: PRM2, ODF1, TP1, ACTL7B and found large reductions in mRNA levels in all patients with spermatogenic defects except patients with idiopathic oligozoospermia. Patients with idiopathic azoospermia had similar levels of gene expression as those carrying Y chromosome microdeletions independently from their SCOS or HS phenotype, suggesting a possible link of the selected genes to a common pathway leading to infertility and that variability of the histopathology is related to other genetic and environmental factors. Further investigations revealed that DAZ gene was not expressed in six patients without the AZFc deletion in the peripheral blood as expected. Testis biopsy was obtained from patients with obstructive azoospermia (10 normospermic controls) and infertile male (10 with Y microdeletions and 10 with idiopathic infertility) with the histopathology ranged from SCOS to severe hypospermatogenesis

P04.22
Association of b2/b3 deletion with spermatogenic failure

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The b2/b3 deletion removes 1.8Mb of the AZFc region on the Y chromosome, including 12 members of 8 testis-specific gene families.

It was suggested that geographical and ethnic differences might affect the Y chromosomal background and phenotypic expression of this deletion. The association of the b2/b3 deletion with male infertility has been reported in Chinese men, whereas no predisposition was detected in several other populations. The b2/b3 deletion is found fixed in the Y haplogroup N which is widely distributed among men in the Northern Europe. It is present with low frequencies among other European populations. We studied the occurrence of partial AZFc deletions among 77 men with azoospermia, 93 with oligozoospermia, 44 normozoospermic individuals with unexplained couple infertility and 233 fertile controls (proven fathers). The methodology included analysis of AZFc specific STS markers, DAZ and CDY gene dosage and single nucleotide variants. The Y chromosome haplogroups were determined by 28 Y-chromosome SNP markers, which were typed by multiplex PCR/SNaPshot reactions. We found 6 men with b2/b3 deletion, one azoospermic men, 4 oligozoospermic and one fertile control. The b2/b3 deletion was significantly more frequent among men with impaired spermatogenesis (5 out of 170 men or 2,9%) than among fertile controls (1 out of 233 or 0,4%; p=0,04). The highest frequency was present in oligozoospermic men (4,3%; p=0,01). There was a difference in the distribution of the Y haplogroups among males with b2/b3 deletion; all five infertile men belonged to haplogroup E3b1, while the only fertile men belonged to haplogroup N3.

P04.23
High prevalence of two Y chromosomal haplogroups N1c1-M178 and N(xM178) in Russian infertile men with b2/b3 deletion

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An association between Y chromosome deletion b2/b3 and haplogroup N is well defined. The aim of our study was to evaluate a frequency of partial AZFc deletions and to analyze of Y haplogroups in Russian infertile men with b2/b3 deletion.

Materials and Methods: We screened 2933 infertile Russian men for Y microdeletions. Complete AZF deletions were detected according to Laboratory guidelines EAA/EMQN (1999). Partial AZFc deletions were tested by mPCR for STSs: sY142, sY1197, sY1192, sY1291, sY1206, sY1054, sY1125. Y haplogroups were analyzed in randomly selected 158 individuals with b2/b3 deletions. DNA samples have been genotyped by the set of Y chromosomal SNPs to identify the association with Y haplogroups. SNP typing was performed by the custom TaqMan assays on the real-time PCR instrument 7900 (Applied Biosystems).

Results: Partial AZFc deletions were found in 12.1% infertile men. The b2/b3 deletion was detected in 8.6% individuals. The patients with b2/b3 deletions presented with a wide range of spermatogenetic failure, at that severe oligozoospermia to azoospermia were prevalent. About 80% of samples fall into haplogroup N1c1-M178, 10% fall into the related haplogroup N-LLY22 (xM178), and only the rest 10% fall into other haplogroups, including R1a-M198. Note, that 90% of the studied individuals were ethnic Russians, and the obtained frequencies of Y haplogroups drastically differ from the frequencies in the general Russian population.

Conclusion: The b2/b3 deletion is commonest Y chromosome microdeletion in Russian infertile men. We conclude that this deletion exhibit strong association with two related Y chromosomal haplogroups: N1c1-M178 and N-LLY22(xM178).

P04.24
The study of Y chromosome microdeletions and mutations in the androgen receptor gene in Iranian patients with idiopathic non-obstructive azoospermia

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Introduction: Genetic factors, including Y chromosome microdeletions and androgen receptor (*AR*) gene mutations are responsible for male infertility. The aim of this study was to determine the incidence of AZF deletions and the prevalence of *AR* gene mutations among Iranian infertile men with idiopathic non-obstructive azoospermia.

Materials and Methods: A total of 100 Iranian azoospermic infertile men was selected for the molecular study of Y chromosome microdeletions and mutations in the AR gene. The presence of eight sequence tagged site (STS) markers from AZF region was investigated using multiplex PCR. Screening for *AR* gene mutation was performed using PCR-SSCP (Single-strand conformational polymorphism) and sequencing.

Results: 12% of the patients showed Y chromosome microdeletions and among these patients, deletion in AZFb region was the most frequent (66.67%). PCR-SSCP and sequencing analysis revealed a novel 1510C→A transversion in the exon 1 of the *AR* gene resulted in p.Pro504Thr substitution. Analysis of CAG repeats in the exon 1 of the *AR* gene showed a significant statistical difference between the CAG length in the patients and fertile controls (P=0.014).

Conclusion: According to the relatively high incidence of Y chromosome microdeletions among Iranian azoospermic patients molecular screening should be advised to infertile men before using assisted reproductive treatments. Furthermore, the present study indicates that molecular analyses of *AR* gene play an important role in diagnosing the cause of infertility in patients with azoospermia and therefore, may be useful for genetic counseling of candidates for assisted reproductive techniques.

P04.25
Mutation analyses of the TSPYL1 gene in infertile patients with azoospermia, cryptozoospermia or oligozoospermia

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Background: Recently, mutations in the *TSPYL1* gene have been reported to be associated with an autosomal recessive sudden infant death with dysgenesis of the testes syndrome (SIDDT) and with 46,XY disorder of sex development (DSD) and male idiopathic infertility, respectively. However, the exact function of TSPYL1 in testicular development and spermatogenesis is currently uncertain.

Methods: Mutation analysis was performed for *TSPYL1* in a cohort of 106 infertile men with idiopathic non-obstructive azoospermia, cryptozoospermia and oligozoospermia, and in a control group of up to 105 men with proven paternity.

Results: 8 known SNPs were detected in the *TSPYL1* gene of which none was significantly associated with male infertility. One azoospermic man was heterozygous for the yet undescribed missense mutation c.419C>G (p.Ser140Cys). This sequence variation was not observed in 102 fertile men with proven paternity. Additionally, one out of 101 fertile men was shown to be heterozygous for the missense change c.487G>A (p.Val163Ile). The p.Val163Ile variant was not identified in 106 infertile men. We analyzed both missense mutations with the online-softwares “Mutation Taster” (www.mutationtaster.org), “PolyPhen2” (http://genetics.bwh.harvard.edu/pph2/) and “PMut” (http://mmb.pcb.ub.es/PMut/), and none of these programs predicted a disease causing effect for c.419C>G (p.Ser140Cys) and c.487G>A (p.Val163Ile), respectively. These assessments await confirmation by functional studies.

Conclusions: Mutations in the *TSPYL1* gene do not seem to play a major role in the pathogenesis of idiopathic male infertility. Mutation screening of the *TSPYL1* gene can currently not be recommended in diagnostics of idiopathic male infertility.

P04.26
MTHFR C677T and A1298C mutations in couples with infertility problems

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INTRODUCTION. Infertility is a worldwide reproductive health problem that affects approximately 15% of married couples.

Methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C mutations are associated with recurrent spontaneous abortions and may be overrepresented in couples who have fertility problems or have experienced recurrent miscarriages. In this study we have analyzed the MTHFR C677T and A1298C genotype distributions in couples with unexplained fertility problems and healthy controls.
METHODS. DNA was extracted from peripheral blood samples and allele specific PCR was performed for the detection of each mutation. The MTHFR C677T and A1298C mutation frequencies between patient groups and the control groups were analyzed using the Chi-square test. P-values of <0.05 were taken as statistically significant.
RESULTS. Comparison of combined MTHFR C677T/A1298C genotype distributions between probands and controls is presented in table.

Genotype MTHFR C677T/A1298C	Infertility probands (n = 200)	Controls (n = 222)	Female probands (n = 100)	Female controls (n = 111)	Male probands (n = 100)	Male controls (n = 111)
CC/AA	17	21	8	10	9	11
CC/AC	35	49	20	22	15	27
CC/CC	26	24	11	15	15	9
CT/AA	36	45)	17	22	19	23
CT/AC	49	47	25	24	24	23
CT/CC	6	8	3	4	3	4
TT/AA	20	25	11	11	9	14
TT/AC	8	1	3	1	5	0
TT/CC	3	2	2	2	1	0
chi square (all samples vs. controls) 9.25, p = 0.03 (df = 8)						
chi square (female infertility vs. femal controls) 1.33, p = 0.72 (df = 8)						
chi square (male samples vs. male controls) 13.23, p < 0.001 (df = 8)						

CONCLUSIONS. The present finding indicates that significant difference in the prevalence of MTHFR C677T/A1298C mutations can be found in probands with fertility problems when compared with controls without an infertility history. However, only male probands contributed to the association indicating that MTHFR mutation may be a gender specific factor which affects fertility of grown adults. The presence of combined MTHFR C677T/A1298C genotypes highly increased the risk of men infertility. Our presented data highlight the importance of MTHFR mutation screening in couples with unexplained infertility, especially by male adults.

P04.27
Disruption of the gene for zona pellucida binding protein detected by array CGH in male infertility

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The infertility affects 10-15% of all couples. In about 40% of the cases the male factor is reported to be the main reason for unsuccessful fertilization. Although in 70% of male factor infertility the etiology is recognizable, in the remaining 30% the reason is unknown and the male infertility is named idiopathic. In the current study, we selected 14 patients with idiopathic infertility, affected by azoospermia and oligoasthenoteratozoospermia. All patients were subjected to DNA analysis for deletions of Y chromosome and cytogenetic analysis for chromosomal aberrations. After these analyses, the patients without such kind of abnormalities were further analyzed by array CGH.
Cytogenetic analysis revealed two patients with chromosomal mutations - inv(9)(p11;q13) and t(Y;9)(q12.3;q21.1). Other two patients were detected to have deletions of AZFc region of Y-chromosome. Thus, we applied array CGH analysis for the ten patients without chromosomal or Y-chromosome aberrations. Despite of the presence of known polymorphisms, we established copy number alteration in 17q12-17q21.2 - a region, containing the gene for zona pellucida binding protein ZPBP2. This protein plays a crucial role for the proper spermatogenesis. Our results prompted for investigation of this gene and protein as a potential candidate for spermatogenic failure.

P04.28
High incidence of Y-chromosome AZF microdeletions and AZFc region partial deletions among azoospermic/oligospermic males from Western Ukraine

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Genes of AZF regions of Y chromosome play an essential role in spermatogenesis. The objective of the study was to establish frequency and spectrum of Y-chromosome microdeletions and AZFc region partial deletions among idiopathic azoospermic/oligospermic males from Western Ukraine.
Two hundred idiopathic infertile men (100 with azoospermia and 100 severe oligospermia) and one hundred random fertile men, all with karyotype 46, XY from western region of Ukraine were included in the study. DNA of the above samples was isolated using the salting out method and microdeletions of AZF region and AZFc partial deletions were analyzed by multiplex PCR.
AZF microdeletions were detected among 13.0% males with azoospermia (p=0.0001) and 8.0% (p=0.004) males with severe oligospermia: AZFa subregion was deleted in one patient (4.8%), AZFb - 3 (14.3%), AZFb+c - 7 (33.3%), AZFc - 10 (47.6%) As AZFc subregion was the most frequently altered the study of AZFc partial deletions was done. 8.0% azoospermic/oligospermic men without AZF microdeletions were detected to have AZFc partial deletions. The analysis of AZFc partial deletions has shown the presence of AZFc gr/gr deletion among 2.5% persons (mainly with oligospermia), b2/b3 deletions - 4.5% (three with aspermia and six with oligoastenoteratospermia) and absence of sY1291 locus in 1.0% of patients.
No carriers of AZF microdeletions or partial AZFc deletions were identified among control group of fertile men. The differences in Y-chromosomal partial AZFc deletions frequencies between infertile men (azoospermic/oligospermic) versus group of fertile men are significant (p=0.004) and substantiated AZFc partial deletion testing for practical purposes.

P04.29
Detection of DNA fragmentation in human spermatozoa of patients with asthenozoospermia

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Objective: The aim of the present study was to evaluate levels of sperm nuclear DNA fragmentation in a population of infertile men with asthenozoospermia (low motility) and to compare the results from that of fertile population.
Materials and Methods: Semen samples were collected from 30 asthenozoospermic infertile men selected from couples attending the infertility clinic with a history of infertility ≥ 1 year and 30 healthy fertile men with normal semen parameters served as the control group.
After routine sperm analysis according Word Health Organisation (WHO) guidelines, DNA fragmentation was determined using the terminal desoxynucleotidyl transferase-mediated deoxyuridine triphosphate biotin nick-end labeling (TUNEL) assay. Approximately 500 cells were counted, and the percentage of spermatozoa with fragmented DNA (DFI) was calculated.
Results: There are no significant differences in the mean of participant's age, sperm concentration, normal sperm morphology in two groups but the mean of sperm motility of fertile males was significantly higher than that of asthenozoospermic males.
The difference was not significant between the percentage of DNA fragmentation in patients with asthenozoospermia and the normozoospermics men (9.46 ± 8.68 and 8.19 ± 6.84). In addition, no significant correlation was found between the degree of asthenozoospermia and the DFI value in the asthenozoospermic group, when this group was subdivided into moderate, severe, and extreme asthenozoospermia.
Conclusion: Asthenozoospermia was not associated with an increase of DNA fragmentation. So impairment of sperm motility is not a critical parameter for analysis of sperm DNA damage.

P04.30
Evaluation of *SHOX* duplications in Finnish patients with Müllerian aplasia

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Duplications of the short stature homeobox (*SHOX*) gene have been reported in some patients with Müllerian aplasia (MA), a reproductive disorder presenting as congenital absence of uterus and upper two third of the vagina. We have performed MLPA (Multiplex Ligation-dependent Probe Amplification) studies in a cohort of 98 Finnish patients with MA (sporadic, N=96 and familial, N=2) and 118 healthy women in order to identify *SHOX* duplications. A commercial kit (SALSA MLPA kit PO18-E1 SHOX, MRC Holland) including probes for each exon of *SHOX* as well as probes for regulatory areas of the gene were used in the analysis. All patients and controls showed normal amplification of *SHOX*. Aberrant amplifications for regions downstream of *SHOX* were detected in a few patients and in some of the healthy controls. However, these observed copy number changes were reported in the Database of Genomic Variants (http://projects.tcag.ca/variation), indicating that these were harmless polymorphisms. Our MLPA results from a large cohort of patients with MA suggest that duplications of *SHOX* are not a frequent cause of the disorder. Further genetic studies are needed to find out the underlying cause(es) for MA.

P04.31
The Association between LH, FSH, Testosterone hormones' serum levels and prevalence of gr/gr, b1/b2, b2/b3 polymorphisms in Iranian oligozoospermia males

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Introduction: Infertility is a main healthy problem. About 10-15% of couples suffer this problem and in the 50 %, the male factor affects. The human Y chromosome contains essential genes for spermatogenesis specially those which are located on four major intervals defined as AZFa, AZFb, AZFc and AZFd. Partial deletions of the AZFc region is reported as a significant risk factor for oligo-/azoospermia. The main purpose of this study is to investigate the association between Testosterone, FSH, LH serum levels and the prevalence of partial deletions in the AZFc region (gr/gr, b1/b2, b2/b3) in Iranian oligozoospermia males.

Material and methods: Multiplex PCR technique and agarose gel electrophoresis were used to assess complete and partial deletions in 30 oligozoospermia infertile and 50 fertile men, and Levels of hormones were measured by Imunoenzymatic kit (EIA) in all samples.

Results: 3.3% of the patients showed AZFb deletion but no microdeletion was detected in the control samples. In AZFc region, 17% patients showed deletions, in which 13.7% had gr/gr and 3.4% had b2/b3 deletions. 12% of the healthy individuals showed partial deletions, including gr/gr (10%) and b2/b3(2%). No significant correlation was detected between the presence of gr/gr microdeletion and the testosterone serum level (p=0.856). However, the serum levels for FSH and LH were significantly higher in patients with gr/gr microdeletion (p=0.0001, p=0.0001 respectively).

Conclusion: The present study suggests that applying molecular investigation in oligozoospermia patients with increased serum levels for FSH and LH may be informative before using assisted reproductive treatments.

P04.32
Pleiotropic effects of human Growth Hormone/Chorionic Somatomammotropin (hGH/CSH) genes on the fetal growth and maternal metabolism

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The hGH/CSH locus at 17q22-24, consisting of a pituitary-expressed GH1 and four placenta-expressed genes GH2, CSH1, CSH2, and CSHL1, is implicated in regulation of postnatal and intrauterine growth, and in the maternal metabolic adaptation to pregnancy.

We hypothesized that the mRNA expression profiles, including alternative splice-products of placental hGH/CSH genes contribute to the determination of the birthweight and normal course of pregnancy. To address this question we developed a sensitive, fluorescently labeled semi-quantitative RT-PCR assay coupled with gene-specific restriction resolved on an ABI capillary electrophoresis. The detailed profile of alternative transcripts of GH2, CSH1, CSH2, and CSHL1 genes in placental samples of REPROMETA collection from uncomplicated term pregnancies was determined in association with the birthweight of newborns, grouped as appropriate-for-gestational-age (AGA; n=23), small-for-gestational-age (SGA; n=15), and large-for-gestational-age (LGA; n=34), and by maternal pregnancy-related conditions of pre-eclampsia (PE; n=17) and gestational diabetes mellitus (GD; n=11).

The majority of pregnancies with SGA newborn show down-regulation of the entire hGH/CSH cluster in placenta, while in case of LGA the expression of CSH1-1, CSH2-1 and CSHL1-4 mRNA transcripts in placenta is significantly increased compared to AGA newborns (P<0.0001, P=0.009, P=0.002, respectively) (Männik et al., 2010, J Clin Endocrinol Metab. 95:2433-42). Similarly, the entire hGH/CSH cluster shows reduced expression in placentas of PE compared to uncomplicated pregnancies. In case of GD, the individual genes in hGH/CSH cluster demonstrate differential expression pattern compared to uncomplicated pregnancies.

Our results show that the expression level of placental hGH/CSH genes correlates with fetal growth and may influence maternal metabolism.

P04.33
Clinical relevance of SF-1 (NR5A1) gene mutation screening in primary ovarian insufficiency

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Introduction: Steroidogenic Factor-1 (SF-1), encoded by the *NR5A1* gene, regulates the transcription of multiple genes associated with steroidogenesis, sexual development and reproduction. Recently *NR5A1* mutations were found to be associated with sporadic and familial primary ovarian insufficiency (POI). The high mutation rate in the previously published paper prompted us to further establish the significance of *NR5A1* mutations in a large cohort of women with POI.

Materials & Methods: In 2005 a nationwide phenotype and genotype study on POI was initiated in the Netherlands; all study cases were included for the current study. The entire coding region (exon 2-7) and splice sites of the *NR5A1* gene were PCR-amplified and sequenced. The pathogenicity of identified mutations was determined with splice site involvement, Align-GVGD class and Grantham scores.

Results: 383 Women with POI were included, 77 of them had a family history for POI. Adrenal dysfunction was excluded in all women. Five non conservative missense mutations (mutation rate 1.3%) in 3 sporadic and 2 familial cases of POI were found. Grantham variations were 29, 64, 98, 99, and 113. Splice sites were not involved. Align-GVGD classes ranged from C0 to C25, indicating low to intermediate pathogenicity.

Conclusions: The prevalence of *NR5A1* mutations in a large cohort of women with POI (1.3%) is lower than published previously. Furthermore, low to intermediate pathogenicity was predicted for these mutations. There seems to be limited value for the inclusion of SF-1 gene mutation screening in the diagnostic work-up for women with POI.

P04.34
The frequency of DNA- repair and cell cycle control polymorphisms and pregnancy

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Recently increasing attention is paid to the study of allelic polymorphism of genes related to gene regulatory network of pregnancy pathology. The aim of this work was to study the frequency of polymorphic genes of DNA-repair APEX 1 (APE 1) (Asp148Glu), XPD (Lys751Gln), XPG (Asp1104His), XRCC 1 (Arg194Trp), hOGG 1 (Ser326Cys) and cell cycle control CHEK 2 (1100delC) in women with physiological (control group) and pathological (miscarriage) pregnancy. The investigated material included samples of the endometrium (E), chorion (Ch) obtained after medical or spontaneous abortion, as well as blood (B), taken from the cubital vein.

In reviewing the study groups for the presence of a combination of several unfavorable genetic factors revealed that in women with recurrent miscarriages 3.1% (Ch), 3.7% (E) were simultaneously heterozygotes for five genes, which were not observed in the control group. Moreover, in the group with pathological pregnancy the number of heterozygotes for the four genes simultaneously increased: 18,7% (Ch), 18.5% (E) 5,9% (B) (5.9% (Ch), 16,7% (E) 0% (K) in the control group, respectively). It is important to note that in the group with the pathology of pregnancy 6.3% (Ch), 3.7% (E) were homozygous for polymorphic alleles of two genes simultaneously - APEX 1 and XPD, 13,6% (B) - homozygotes for polymorphic alleles of three genes simultaneously - APEX 1, XPD, hOGG 1.

Analyzing the data, it was found that the combination of certain genotypes in polymorphic loci of DNA-repair and cell cycle control genes may influence on susceptibility to reproductive pathology.

P04.35
Risk assessment of ART and its related factors in the development of Prader - Willi syndrome

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To examine whether assisted reproductive technology (ART) and its related factors can be a risk factor for the development of Prader-Willi syndrome (PWS), we studied 74 Japanese PWS patients who were born during the years 1997-2008 when statistical data on ART are available in Japan.

Of the 74 patients, seven were born after ART (six after ICSI and one after IVF). Molecular studies revealed underlying genetic causes in all patients, including the seven patients born after ART of whom two had deletion of the paternally derived 15q imprinted region and five had trisomy rescue type upd(15)mat (TR-upd(15)mat). The maternal ages at birth were increased in PWS patients conceived after ART (36-45 years), especially in those with TR-upd(15)mat (38-45 years), consistent with the notion that the advanced maternal age is a risk factor for the development of TR-upd(15)mat.

The frequency of births after ART was significantly higher in PWS patients than in the general population ($P=2.2\times10^{-9}$), even after adjusting maternal ages (≥35 years) ($P=5.4\times10^{-6}$). Among PWS patients, however, although the relative frequency of TR-upd(15) mat was significantly higher in patients conceived after ART than in those conceived naturally (P =0.008), it became non-significant after adjusting maternal ages (≥35 years) between the two groups (P=0.22). The results imply that ART and/or ART-related parental and environmental factors can be a risk factor for PWS in general. In addition, the relative predominance of TR-upd(15)mat in patients born after ART is primarily ascribed to an ART-related maternal age factor rather than ART itself.

P04.36
Laeverin expression and its role in invasion and migration of human trophoblast cells: Possible implications in the pathogenesis of preeclampsia

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We reported previously that mRNA levels of laeverin, a membrane-bound aminopeptidase, are 10-fold up-regulated in placentas from severely preeclamptic women compared to their healthy controls. In the present study, using immunofluorescence of tissue sections and tissue arrays, we demonstrate a shift in laeverin protein expression from the cell membrane in healthy trophoblast to cytosol in preeclampsia. Electronmicroscopy indicated that laeverin is retained in the Golgi apparatus in preeclamptic placentas. Additionally, we tested the hypothesis that *laeverin* gene-silencing reduces the invasiveness of the cell line HTR-8/SVneo trophoblasts through a basement membrane-like matrix (Matrigel). Trophoblast invasion was reduced by 58% in *laeverin* knock-out cells compared to controls. Wound healing assay demonstrated reduced migration of trophoblast cell line transfected with siRNA against *laeverin*. We further performed gene expression profiling using RT2 Profiler TM PCR Array in order to find the molecular pathways that might be affected by *laeverin*-silencing. Among the 84 genes present on the array, three genes were significantly (>4-fold) down-regulated: *integrin alpha-2* (39-fold), *matrix metalloproteinase 1* (36-fold) and *integrin beta-3* (5-fold). In conclusion, laeverin is up-regulated in the preeclamptic placenta both at transcriptional and protein level. It appears to regulate trophoblast invasion by affecting cell adhesion molecules and degradation of the extracellular matrix and thus might have a role in the pathogenesis of preeclampsia.

P04.37
A simple approach for detecting novel short-tandem repeat (STR) markers for preimplantation genetic diagnosis

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Introduction: Misdiagnosis in PGD occurs mainly due to allele drop-out (ADO), but can be prevented by the use of flanking polymorphic markers (most commonly CA-repeats). The reliability of diagnosis depends on the number, heterozygosity and informativity of such markers. When database search yields insufficient results, it is essential to discover novel markers.

Aim: To describe a simple approach for detecting novel locus-specific polymorphic markers.

Material and Methods: To detect CA-repeats, we copy the sequence of interest spanning 1 Mb upstream and downstream from the mutation site into a MS Word file. Then we search this sequence using the Ctrl-F function for (CA)n or (TG)n, (where n>10). Such STRs will often be polymorphic. Primers are then designed for each site. A marker is considered informative when the CA repeat size is different among the various alleles in family members.

Results: Over the last 8 years we performed PGD for 65 different disease genes, using 545 flanking polymorphic markers. Of these, 344 (63.1%) were obtained using published data. However, for more than 20 disease genes, with insufficient informative markers, we were able to detect an additional 201 (36.9%) novel CA-repeats using this approach. More than 90% of these sites indeed proved to be polymorphic. This allowed the use of at least 4 informative markers for each case of PGD.

Conclusion **:** We demonstrated a simple approach for search of novel informative CA-repeats that allows maximizing accuracy in PGD and prevention of ADO. This approach is now routinely used in our PGD lab.

P04.38
Deduction of meiotic segregation patterns by pachytene shape analysis in PGD for translocation carriers

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Introduction**:** In translocation carriers, meiosis produces different segregation variants. Most studies of segregation variant frequencies were obtained from observations of viable probands. PGD provides a unique insight into this phenomenon.
Aim**:** Deduction of meiotic segregation patterns as assessed in embryos of translocation carriers undergoing PGD.
M&M: Ninety PGD cycles were performed for 35 translocation carriers. Two blastomeres were aspirated from each embryo for FISH analysis. The resulting segregation variants were deduced. Observed frequencies of different segregants were compared to predicted frequencies, based on pachytene shape analysis, taking into account the length of the centric versus the translocated fragments**.**
Results: Overall 583 embryos were analyzed: 441 from reciprocal translocation carriers and 142 from Robertsonian translocations. In reciprocal translocations, alternate segregation (balanced) was seen in 23.1% of embryos, whereas most were unbalanced (adjacent-1 in 29.4%; adjacent-2 in 5.9%; 3:1 segregation in 25.6%; and 16% had other anomalies).The overall incidence of unbalanced embryos was 71.4%, but was significantly higher in reciprocal translocations. In most translocations, adjacent-2 segregation was rare, but when present, the rate of balanced gametes was lower. There was no significant difference in the rate of unbalanced embryos between female and male carriers. Finally, the distribution of segregation modes was predicted correctly in 82%.
Conclusions: Translocation carriers have a significantly high frequency of unbalanced embryos, more so among reciprocal translocation carriers. In PGD, the frequencies of the different segregation patterns can be predicted by pachytene shape analysis. Some translocations predispose to a higher rate of unbalanced gametes, as compared to others.

P04.39
Preimplantation genetic diagnosis for spinal muscular atrophy at the blastocyst stage

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Background: Approximately 94% of spinal muscular atrophy (SMA) cases are caused by common homozygous absence of the *SMN1* gene. Although preimplantation genetic diagnosis (PGD) is an alternative option for couples at risk of having a child with single gene disorder, blastomere biopsy has been employed in most *in vitro* fertilization procedures.
Methods: In the present study, we validated and applied protocol clinically for PGD through the use of blastocyst biopsy, whole genome amplification, mini-sequencing genotype coupling with genetic linkage of *SMN* gene involving three informative microsatellite markers, and thawed embryo transfer. We report data to identify the *SMN1* gene deletion on eighteen clinical embryos obtained from one participating couple, where both partners are heterozygous SMA carriers with 1-*SMN1*/3-*SMN2* genotypes.
Results: Approximately 78% (14/18) of blastocysts were successfully amplified in a single PGD cycle. Among these embryos, ten (72%, 10/14) were diagnosed as unaffected, two (14%, 2/14) as affected, and two embryos (14%, 2/14) had no conclusive diagnosis due to allele drop-out (ADO). Two unaffected embryos were thawed and transferred in the next cycle resulting in a singleton pregnancy, and the birth of a healthy girl who carries the 1-*SMN1*/3-*SMN2* genotype.
Conclusions: The strategy of PGD using blastocyst biopsy and thawed embryo transfer increased the reliability of the results and permitted more time for the execution of molecular diagnosis. The improved protocol for PGD could be adopted for other monogenic diseases in the future.

P04.40
Preimplantation genetic aneuploidy screening by fluorescent in situ hybridization

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Preimplantation genetic diagnosis (PGD) is a method used to identify genetic defects in embryos created through intracytoplasmic sperm injection (ICSI) or in vitro fertilization (IVF). Single gene defects, hereditary chromosomal disorders (especially couples with translocations carriers) and aneuploidy screening are the most frequent indications for PGD. The aim of the study is to evaluate the incidence of embryo aneuploidy and pregnancy outcome rates. Thirty-three ICSI/IVF patients with various indications who referred to Assisted Reproductive Techniques Unit of Department of Obstetrics and Gynecology were included in the study between 2008-2010 years. Biopsies are performed in their 3rd day embryos with 6-8 blastomers and aneuploidy screening was performed by fluorescent in situ hybridization (FISH) method on the same day. Aneuploidy screening for chromosomes 13, 16, 18, 21 and 22 were analyzed except low grade Turner mosaicism cases. In these cases X and Y chromosomes were analyzed instead of chromosomes 16 and 22. Blastomere biopsy was performed on 165 embryos, which had grade I or II quality. The indications for PGD were as follows; history of a recurrent implantation failure (no: 17, 51.5%), low grade Turner mosaicism (no: 10, 30%), recurrent abortion (no:5, 15%) and Klinefelter syndrome (no: 1, 3.5%). Embryo transfer was performed in 23 patients after PGD, chemical and clinical pregnancy rates were 3 (13%) and 8 (34%), respectively. In conclusion PGD seems to be an effective method in ICSI/IVF patients with chromosomal anomalies.

P04.41
Health of babies born after Preimplantation Genetic Diagnosis; results of a multi-centre retrospective study piloting a parental questionnaire

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Follow up of babies born after preimplantation genetic diagnosis and screening (PGD/PGS) is recommended and international collaboration is required as the number of PGD babies born is low. A pilot parental postal questionnaire was developed to retrospectively collect information about treatment cycles, pregnancy, birth, longer term child health and development. An open source relational database was constructed using MySQL.
Six European PGD centres provided data on 401 deliveries; 112 PGD, 288 PGS cycles and one for both. Between December 1999 and October 2007 493 babies were born: 307(62.3%) singletons, 180 (36.5%) twins & 6(1.2%) triplets (2 not known). Mean birth weights were 3.17, 2.55 and 1.67kgs respectively. Eighty one percent of babies were born at ≥36 wks, 14.5% between 30-36 wks and 3.2% ≤ 30 wks gestation. Neonatal problems occurred in 129 babies (26%), 113 babies (22.9%) requiring special care. Nine abnormalities were recorded in 7 babies (1.42%) included tongue tie, cardiac anomalies, hip dysplasia, talipes, hypospadias & undescended testes. Since birth 378 (76.7%) babies were recorded as having no health problems.
The study allowed the development of standardised nomenclature for data capture which used in a web based format, could provide easy access to a wide demographic population for prospective data collection. The birth abnormality rate in this cohort is low at 1.42%. However 22.9% of PGD infants required special care and the incidence of health problems since birth was 23.3%. Further analysis of these health problems is required to determine major & minor rates.

P04.42
Ten years experience of prenatal diagnosis and preimplantation genetic diagnosis for Huntington disease in the Netherlands

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INTRODUCTION: In the Netherlands prenatal diagnosis (PND) for Huntington disease (HD) is available since 1987. Preimplantation genetic diagnosis (PGD) was first applied in 1999. For this study we surveyed all couples referred for PND and/or PGD for HD between 1996 and 2008.
METHODS: Information on couples' reproductive history, pregnancies with/without PND, PGD cycles, and follow up of all subsequent pregnancies was included until December 2010.
RESULTS: 184 couples were surveyed. For the first pregnancy 59% opted for PND, 14% started PGD, 13% had their first child untested, 11% refrained from PGD after intake and had no PND in subsequent pregnancies, 3% had a miscarriage. In 79.9% of couples one partner was a known carrier (61 males/86 females). In 20.1% one of the partners was at risk (26 males/11 females).
In subsequent pregnancies, 29 couples started PGD (24 after a history of PND and/or miscarriage). 22 couples secondarily underwent PND (19 after untested pregnancies, 3 after ≥1 PGD cycles). 68 couples applied for PND in ≥ 2 subsequent pregnancies.
In total 132 couples performed PND in 266 pregnancies (86% direct testing, 14% exclusion testing): more details will be provided.
PGD was performed in 51 couples (1 exclusion PGD): 123 cycles resulted in 25 deliveries (23 couples).
CONCLUSIONS: According to the alternating use of PND and PGD we conclude that couples reconsider their choices in every subsequent pregnancy based on their previous experience, personal beliefs, and probably time pressure.

P04.43
Preimplantation genetic diagnosis for chromosomal rearrangements: results of 2000 embryos

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Preimplantation genetic diagnosis is a technique used to identify genetic defects in embryos created through in vitro fertilization (IVF) before transferring them to the uterus. Carriers of chromosomal rearrangements are one of the major group of patients who get benefit from this technique. Here we present our results for PGD performed for translocation and inversion carriers. From 2001 to 2011, 213 patients underwent PGD following 304 assited reproductive treatment (ART) cycles. 136 patients were carrying reciprocal translocation (RecT), 63 patients were carrying robertsonian translocation (RobT), 1 patient was carrying both reciprocal and robertsonian translocation, 1 patient was carrying a complex translocation and 12 patients were carrying inversions. Blastomeres or trophectoderm tissues were fixed with hypotonic solution/3:1 fixative method.Commercially supplied telomeric,locus specific and centromeric FISH probes were used. For cases done in years between 2007 and 2011, aneuploidy panel (13,16,18,21,22) was also included in the PGD study. From a total of 2000 biopsied embryos, 90% were diagnosed. For RecT carriers 197 cycles, for RobT carriers 90 cycles and for inversion carriers 14 cycles were performed. The clinical pregnancy rates were 36.8%, and 31.1% respectively. For RecT carriers 20.3% and for RobT carriers 34.7% of embryos were found to be normal or balanced. It was found that; the gender of the translocation carrier, maternal age and the type of the translocation affected the percentage of normal or balanced embryos. To date, 59 healthy babies have been born for translocation and inversion carriers.

P04.44
Preimplantation diagnosis of monogenic diseases in GENNET

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In 2007 we completed the first in vitro fertilization cycle (IVF) followed by preimplantation genetic diagnostics (PGD) of cystic fibrosis. Since than, we have performed over 120 cycles with scheduled PGD for 43 different monogenic diseases. The list of diagnosis where we can offer PGD is being continually extended.
In our center we use genetic haplotyping technique by multiplex PCR technique on products of MDA (multiple displacement amplification) from 1 blastomere biopsied from the cleavage-stage embryo.
In rare cases of unambiguous results from the blastomere, analysis can be repeated from trofoectoderm and embryo is still managed to be transfer on the day 5. We have been working on expanding of PGD cases resolvable in our center. This year we successfully completed first cycle of male partner de-novo mutation case based on sperm-haplotyping analysis supplemented by proof of mutation by sequencing analysis.
Up to now, we have accomplished 120 IVF cycles with PGH. In average 7-8 embryos were biopsied in one IVF cycle, as preferably mild stimulation protocols were applied.
We completed accreditation of the method by ISO regulation 15189:2007 in 2009. We annually participate in EQA organized by UK NEQAS.
PGD represents an alternative within prenatal diagnosis services and so offers a chance for many couples in risk of genetic disease to have a healthy baby and to avoid the risk termination of the pregnancy from genetic reasons or birth of affected child. Currently available PGD technology can help eliminate some genetic diseases in the future.

P04.45
PRM1 and PRM2 gene polymorphisms in Czech men with idiopathic oligozoospermia, normozoospermic men and men with proven fertility

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The most frequent ACC haplotype formed by PRM1 230A>C and PRM2 298G>C/373C>A variants was associated with increased sperm counts in German males [1]. The aim of our study was to verify this impact on spermatogenesis in Czech males with idiopathic oligozoospermia.
PRM1 and PRM2 sequencing was performed on 3130xl Genetic Analyzer in 52 men with idiopathic oligozoospermia, in 52 normozoospermic and in 75 males with proven fertility.
In PRM1 we detected one common variant (230A>C) with an overall minor allele frequency (MAF) of 28.5% and three rare variants (c.54G>A, c.102G>T and c.166C>T) with overall frequencies of 0.56%, 0.28% and 0.28%, respectively. In PRM2 we detected two common polymorphisms (298G>C and 373C>A) with overall MAFs of 49.1% and 29.3%, respectively, and two rare variants (c.201C>T, c.377C>T), both with overall frequencies of 0.28%.
The prevalence of all detected variants and the ACC haplotype between men with idiopathic oligozoospermia and controls was not significantly different. The rare protamine gene variants are also not directly related to disorders of spermiogenesis in Czech males as previously described in German males [1]. In contrast, we have not confirmed any relationship of the ACC haplotype with sperm concentration. Our results indicate that the impact of PRM1 and PRM2 gene variants on increased sperm counts is dependent on population genetic background and/or epigenetic impacts of different environment and lifestyle factors.
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P04.46
Preimplantation Genetic Diagnosis in female and male carriers of Reciprocal Translocations: clinical outcome of 312 cycles.

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Introduction: Carriers of Reciprocal Translocations (RecT) are at risk for reproductive problems. For some carrier couples Preimplantation Genetic Diagnosis (PGD) could be an option to fulfil their child wish.
In this study we retrospectively analyse the results of PGD for RecT at our centre.
Materials and methods: An observational study was performed of all cycles for PGD for RecT at a tertiary referral centre between January 1997 and December 2007.
Data on patient characteristics and cycle outcomes were registered.
Results: During a period of 11 years 312 PGD cycles were performed for 69 male and 73 female carriers of RecT. The mean female age was 32.8years, the mean male age 35.8 years. Most carriers were diagnosed with a translocation because of fertility problems or recurrent miscarriages and most of them opted for PGD to avoid these problems.
In 150 of the 312 cycles, embryo transfer was feasible and 40 women delivered a healthy singleton or twin. This gives a live birth delivery rate of 12.8% per started cycle and of 26.7% per cycle with embryo transfer.
Conclusion: Due to the large number of abnormal embryos, PGD cycles for RecT often lead to cancellation of embryo transfer, explaining the low success rate when expressed per cycle with oocyte pick-up.
Once embryo transfer was feasible, the live birth delivery rate was similar to that of PGD in general at our centre.
PGD is therefore an established option for specific RecT carriers and its availability should be discussed during reproductive counseling.

P04.47
Multiple epimutations of imprinting genes in early pregnancy loss

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Genomic imprinting plays a critical role in regulation of fetal development. Previously we have reported a tissue-specific loss of methylation in KCNQ1OT1 and PLAGL1 in 9.5% and 10.3% spontaneous abortions, respectively. The aim of the present research was identify DNA methylation levels of 115 differentially methylated sites of 52 imprinting genes in samples of first-trimester spontaneous abortions using GoldenGate Methylation Cancer Panel I (Illumina, USA). We have analyzed cytotrophoblast (CT) and extraembryonic mesoderm (EM) from 13 first-trimester spontaneous abortions (8.3±1.8 weeks) with normal karyotype and 4 induced abortions (7.8±0.7 weeks) as a control group. Fourteen (27%) imprinting genes have revealed abnormal methylation in most miscarriages. Eight of these genes (PEG10, GRB10, CPA4, PHLDA2, WT1, ZNF215, HTR2A, GABRB3) were hypomethylated, whereas 6 genes (DCN, TRPM5, H19, INS, GABRA5, PWCR1) were hypermethylated. Significantly, epimutations were found in both tissues (22%) or confined by EM (65%) or CT (13%). Presence of hypomethylation in both tissues indicates reprogramming errors in the primordial germ cells during gametogenesis or at early stages of preimplantation development. The hypermethylation of imprinted genes in both tissues can be a result of reprogramming errors during gametogenesis. Whereas, tissue-specific epimutations allows suggesting independent sporadic epigenetic errors in imprinting maintenance in derivates of various embryonic germ layers after its divergence. Our results provide first evidence for the multiple germinative and somatic epimutations of CpGs within imprinting genes, which can be responsible for the dysfunction of imprinted loci during early embryo development. This study was supported by Federal Program (contracts P303 and P806).

P04.48
Locus copy number variation (CNV) may contribute to recurrent miscarriage

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Recurrent miscarriage (RM; ≥3 miscarriages) is a heterogeneous condition occurring in 1-2% of fertile couples. Despite a wide spectrum of known causes and mechanisms leading to RM (disruptions of thrombophilic, immunologic or endocrine pathways), 50% of the cases are defined as idiopathic. The aims of the current study were (1) to explore whether gene copy number variations (CNV) may contribute to inheritable predisposition to RM, and (2) to identify novel loci involved in establishing a successful pregnancy and to provide new insights into the mechanism of RM.
In total, 73 Estonian samples (43 RM patients and 30 fertile) were analyzed on Illumina Infinum genome-wide genotyping platform. In order to predict the presence of a CNV, two algorithms (PennCNV and QuantiSNP) were used in parallel to analyze the genome-wide data. For twelve genomic loci number of gene copies in each discovery individual (n=73) was experimentally validated by TaqMan qPCR. Six validated CNV regions were selected for the replication experiment in an independent sample (Estonians; n=119 RM patients and n=128 controls).
In the Estonian discovery and replication samples, three tested rare CNVs (MAF <10%, 25-52 kb) showed concordant allele frequency differences between cases and controls. Two of these CNVs cover loci, which have been previously shown to function in the establishment of a successful pregnancy - regulation of trophoblast invasiveness or immunological response. In order to confirm the impact of the identified CNVs in susceptibility to recurrent pregnancy loss, experiments for the replication of these initial findings in other populations are ongoing.

P04.49
Diagnostic value of a-CGH method for recurrent miscarriage and implantation failures

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INTRODUCTION: Endometrial and tubular problems, defective embryo, genetic factors and environmental impacts are among the reasons for recurrent miscarriages and implantation failures. A-CGH is a new method to investigate these impacts.A-CGH can detect chromosomal abnormalities in a reliable way and this technology compares the copy number of segments in two different genomes.
MATERIAL AND METHOD: Whole genome scan was performed on 50 patients admitted to Kocaeli University Medical Genetics Laboratory with the prediagnosis of recurrent miscarriage and implantation failure. DNA of the patient was hybridized on 4x44K microchips as per the protocol. Microarray scanner (Agilent Microarray Scanner; Agilent Technologies, Palo Alto, CA) was used for scanning. The data was pooled in Agilent Feature Extraction software and normalized using CytoSure Analysis Software v.2.0.8.
RESULTS: In our study, various genetic aberrations were observed in 4 patients out of 50 who underwent whole genome scan with a-CGH method. Array-CGH results are as follows: in Xp22.31 region deletion of 1.5 Mb; and the following duplications were detected: in 17q12 region 1.383 Mb, in 20p12.3 region 1.37 Mb, between the bands 4q12 570 Kb.
CONCLUSION: Conventional cytogenetic methods help by 5-8% and a-CGH method helps by 10% in the diagnosis of recurrent miscarriage and implantation failures. A-CGH technology could be a new diagnostic opportunity to screen recurrent miscarriage and implantation failures.

P04.50
Recurrent Pregnancy Loss and Its Relation to Combined Parental Thrombophilic Gene Mutations

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Background and Aim: Recurrent pregnancy loss (RPL) is a heterogeneous disorder that has been associated with antiphospholipid

syndrome and other prothombotic parameters. The contribution of specific thrombophilic genes to the pathophysiology of RPL has remained controversial. We aimed to investigate the prevalences of 12 thrombophilic gene mutations in RPL couples in the current results. ***Method*** :In the current prospective case-control study, in a total of 543 Turkish women with RPL and 327 of their male partners (870 individuals with RPL) , and a control group of 106 fertile couples (control) were analyzed for FV Leiden, factor V H1299R, factor II prothrombin G20210A, F XIII V34L, β-fibrinogen −455G>A, plasminogen activator inhibitor-1, GPIIIa L33P (HPA-1 a/b L33P), MTHFR C677T, MTHFR A1298C, ACE I/D, Apo B R3500Q, and Apo E genes.***Results:*** The overall, heterozygous and/or homozygous point mutations in FV Leiden - FVR2, ApoE2, PAI-1, MTHFR C677T - A1298C and ACE genes were associated with RPL. There was no meaningful association between RPL and other studied genes. ***Conclusion:*** In contrast to the other point mutations and polymorphisms, the homozygosity of 4G in PAI-1 and MTHFR C677T genes in women with recurrent pregnancy loss, and heterozygosity of FV Leiden, FVR2, ACE, ApoE2 genes in both parents play crucial role in RPL and should be considered as a risk factor in RPL. Current results showed that RPL is related to combined parental (not only maternal) thrombophilic gene mutations.

P04.51
Polymorphisms in the annexin A5 gene promoter in Japanese women with recurrent pregnancy loss

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Recent findings have raised the possibility that polymorphisms within the annexin A5 gene (*ANXA5*) promoter contribute to the etiology of recurrent pregnancy loss (RPL). In our present study, 243 Japanese women who had suffered more than three fetal losses and a group of 119 fertile controls were genotyped for four *ANXA5* gene promoter SNPs (SNP1-4: g.-467G>A, g.-448A>C, g.-422T>C, g.-373G>A) previously reported to be associated with this disorder. Additional two SNPs located within the 5'-untranslated region of the *ANXA5* (SNP5 and 6: g-302T>G, g.-1C>T) were also evaluated. Our case-control study revealed that the minor allele was significantly frequent in the RPL group for all six of these SNPs, among which SNP5 showed the highest significance (*P*=0.002). As with the M2 haplotype for SNP1-4 (A-C-C-A) suggested for a western population in previous reports, a haplotype comprising all of the minor alleles for SNP1-6 (A-C-C-A-G-T), the third major haplotype in the Japanese population, showed a significant frequency in our current RPL subjects (*P*=0.025). In addition, the second major haplotype (G-A-T-G-G-C) was also found to confer a significant risk of RPL (*P*=0.036), implicating SNP5 as a major risk determinant for this disease. Our present findings support the hypothesis that genomic variations within the *ANXA5* gene upstream region impact upon the disease susceptibility to RPL. Our data also indicate that SNP5 is a novel risk factor for this disease in the Japanese population.

P04.52
Cytogenetic, Y chromosome microdeletion, and sperm DNA integrity analysis in male partners of couples experiencing recurrent spontaneous abortions

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Couples experiencing recurrent spontaneous abortions (RSA) undergo severe physical and emotional stress, where the aetiology in majority of (60%) cases is still unknown. The aim of the study was to find the role of cytogenetic, Y chromosome microdeletion, and sperm DNA fragmentation in male partners of couples experiencing RSA. Forty eight couples with history of RSA and 20 fertile controls were included in the study. The study subjects were divided into male partners of RSA couples with abnormal sperm parameters (SA) (N= 16), male partners of RSA couples with normal sperm parameters (NS) (N=32) and age matched fertile controls with normal sperm parameters (FC) (N=20). Semen analysis, Karyotyping and Y chromosome microdeletion analysis were carried out as per standard protocol. Sperm DNA damage was measured by flow cytometry and expressed as DNA fragmentation index (DFI). One of 48 RSA men (2%) showed 46, XY (1qh-) chromosomal complement. None of the subject showed Yq deletions. Sperm count was found be significantly lower in SA cases compared to group NS cases ($p<0.0001$) and FC ($p<0.005$). Male partners of RSA couples with abnormal and normal sperm parameters had higher sperm DNA damage ($P<0.0001$). Other than chromosomal anomalies, sperm DNA fragmentation may be the underlying pathology in RSA, thus screening for DNA fragmentation has diagnostic capabilities. However, Yq microdeletion screening in male partners of RSA couples has no diagnostic or prognostic value.

P04.53
Paternal factors: sperm oxidative stress and DNA damage in idiopathic recurrent spontaneous abortions

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Aim: The most common problem in couples attempting pregnancy is recurrent spontaneous abortions (RSA). In majority of the couples experiencing RSA, the causes are unknown. This study was planned to analyze seminal oxidative stress (OS) as well as the level of DNA damage of sperm in couples with history of idiopathic RSA.
Materials and Methods: Twenty two (22) couples experienced RSA and 30 fertile controls were included in the study. OS were evaluated by assessing ROS levels in the semen by chemiluminescence assay and were expressed as RLU/min/20 million sperm. For analysis of the DNA fragmentation, SCSA (Sperm chromatin structure assay) was used as it delivers a highly accurate measure of fertility potential.
Results: RSA and control groups did not show any significant differences in the sperm parameters. However, the mean seminal reactive oxygen species levels were found to be significantly higher in RSA group as compared to controls (12629.23 ± 3245.12 Vs 2535.55 ± 135.24). Fourty percent (40%) of RSA group had higher ROS levels (>2535.55) as compared to controls. Sperm DFI in patients was 32.88±7.41% as compared to 22.50±2.8% in controls.
Conclusion: Thus seminal oxidative stress and sperm DNA fragmentation may be one of the causes for unexplained RSA. Thus DFI (DNA fragmentation index) and OS could be a good clinical prognostic marker and helps to predict outcomes of both assisted and spontaneous conceptions. Treatment with antioxidant can reduce ROS levels and after 3-6 months result in significant decline in sperm DNA damage.

P04.54
Habitual abortion and *de novo* 2q37 deletion

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Background. Habitual abortion or recurrent miscarriage is the occurrence of three or more pregnancies ending in miscarriage, usually before 20 weeks of gestation. Miscarriages frequently occur in the population.
Methods. We describe a couple with recurrent miscarriages and the genetic analyses of placental material from their last spontaneous abortion in first trimester.
Results. Array Comparative Genomic Hybridization (aCGH) identified a *de novo* 2q37 deletion.
Conclusions. The identified mutation and the deleted genes may broaden the clinical spectrum around abortions. ACGH may be the investigation of choice rather than ordinary chromosomal investigations since it is difficult to culture cells from aborted material.

P04.55
Recombination hotspots and preimplantation genetic haplotyping for sickle cell anaemia

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Preimplantation genetic haplotyping (PGH) involves amplification of the whole genome from a single blastomere followed by haplotyping. PGH requires sufficient fully-informative STR markers in close proximity to the gene of interest for any given couple.
Sickle cell anaemia (SCA) is caused by specific mutations in the HBB gene (mainly HbS or HbC). Our experience with PGH for SCA using STR markers indicates that recombination hot spots close to the gene reduce the number of transferable embryos available, due to recombinant haplotypes, which may or may not carry the mutation. The distance between the recombination hotspot and the common mutation is ~30kb; informative markers within this region are therefore necessary in order to diagnose mutation status in all recombinant embryos. Alternatively, identification of a common haplotype harbouring the mutation could be informative for diagnosis.
Novel STRs and SNPs were identified across the region; however, none were found within the 30kb region of interest. Following testing of DNA from 36 members of SCA families and 28 MDA products of single cells from embryos using these SNPs and STRs, a common mutation-carrying HbS haplotype was observed in 9/10 cases. The single exception found means that this approach is unsuitable for definitive PGH diagnosis. An ARMS test was therefore designed for unique identification of HbA, HbS and HbC alleles; this test is now used when a recombination event is observed, for direct determination of the mutation status of the embryo.

P04.56
Spontaneous abortions: "Split's protocol" for successful next pregnancy

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During the last 17 years 534 couples attended Genetic Counseling Unit, Split, Croatia asking for genetic counseling due to the repeated spontaneous abortions (RSA). The couples expect the information about the risk for having a live-born child with potentially serious anomalies, as well as the risk for future miscarriage. Therefore, the objectives of this retrospective case-control study were to find the possible reason(s) for RSA in chromosomally normal parents. Our hypothesis is that the RSA is caused by two hits. The first one is familiar predisposition, while the second is chronic infections. To validate this hypothesis we analyzed the data obtained from couple's medical history and pedigree analysis such as a) personal life-style and habits, b) existence of former urinary and/or genital infections in both partners and their relatives in a first, second and third generation; c) earlier spontaneous abortions (SA), stillborns, other illnesses of both parents and their family members d) SA among the siblings of second generation, e) serological results for EBV, CMV, HSV1/2 and for other viral or protozoa infections, as well as reactivation of latent EBV infection, as well as f) pathohystological status of aborted material, g) former or present urinary and/or genital infection in both partners, h) iron and feritine values. The data confirmed the proposed "two hits" theory for RSA. Based on this "Split protocol" for successful next pregnancy is suggested.

P04.57
Thrombophilic mutations among woman with spontaneous abortions

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Inherited thrombophilia as a genetic risk factor for thrombosis has been implicated in etiology of spontaneous abortions and may contribute to adverse pregnancy outcomes. Maternal thrombophilia augment thrombotic tendency of pregnancy leading to impaired implantation and placentation of the embryo. To determine the association of specific inherited thrombophilias and spontaneous abortions, 10 gene mutations (Factor V Leiden G1691A, Factor V A1299G, Factor II G20210A, Factor XIII G34C, PAI-I -675 4G/5G, FG-ß -455G/A, MTHFR C677T, MTHFR A1298C, MTR A2756G and MTRR A66G) were investigated using multiplex SNaPshot analysis and separation of the fragments by capillary electrophoresis. The prevalence of these thrombophilic markers was studied in 95 women with history of fetal loss (40 of Macedonian and 55 of Albanian ethnicity) and 72 matched fertile controls (37 of Macedonian and 35 of Albanian ethnicity) . Genotype and allele frequencies of the studied mutations were not statistically different between patients and controls and between the two ethnic groups. However, we found higher percentage of thrombophilic mutations in patients than in controls although statistically not significant (≥3 mutations: 92.6% vs. 84.7%, ≥4 mutations: 74.7% vs 69.4%, ≥5 mutations: 52.6% vs 43.1%, ≥6 mutations: 25.3% vs 18.1%, ≥7 mutations: 13.7 vs 8.3%, respectively). These results indicate that thrombophilia might contribute to etiology of spontaneous abortions.

P04.58
Chromosome 21 Copy Number Analysis in Sperm

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The mechanism(s) underlying the low paternal origin of trisomy 21 Down syndrome (less than 10%) is/are still unknown. We have previously suggested that the common maternal origin of trisomy 21 Down syndrome is due to foetal ovarian mosaicism (Hultén et al 2008). In contrast, foetal testicular mosaicism does not seem a likely reason for the rare cases of paternal origin (Hultén et al 2010a; review in Hultén et al 2010b). In order to get to grips with the paternal origin we have now initiated FISH studies to record the copy number of chromosome 21 at various stages of spermatogenesis. We here present the initial data on the chromosome 21 copy number by FISH analysis of spermatozoa from a sample of 5 men with normal spermiograms. It is well known that both false positive and false negative results may be common, when applying a single chromosome-specific probe. We have therefore in this study (as in the previous) applied two 21q probes (Vysis 21q22 and Cytocell 21qtel) and a control chromosome 18 probe for the identification of the copy number of chromosome 21. The disomy frequency was found to be on average 0, 11%, with a range of variation at 0,00 - 0,25% by analysis of 2000 spermatozoa per case. To our knowledge this is the first FISH study using two rather than a single probe for the estimation of the copy number of chromosome 21 in spermatozoa from a control population of men with normal spermatogenesis.

P04.59
The choice of spouse and social homogamy in the Region of Sidi El Kamel (West of Morocco)

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Genes circulate throughout society according to the dynamics established by the types of encounters and associations between individuals who carry these genes.The choice of spouse influences the genetic structure of the family and guides the evolution of the gene pool of the population.
The objective of this work is to study two modes of assortative mating: educational homogamy and homogamy social and professional.
Our study is a prospective study, between January and June 2008, included 490 families in the region of Sidi El Kamel.
The results of this study show quite marked homogamous behavior.
The frequency of consanguineous marriages decreased over the generations, but the decline in the practice marriage is still very low.

J04.01
Chromosomal abnormalities in couples with recurrent abortions and stillborn malformed child

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Cytogenetics studies have an important role in the evaluation of couples with history of recurrent abortions and stillborn malformed child. In order to estimate the frequency of chromosomal abnormalities

in the couples with this kind of reproductive failure we performed a study on 81 couples (162 patients) whose kariotype was established on GTG-banding metaphases from peripheral blood lymphocytes cultures using standard methods.
The frequency of chromosomal abnormalities on our studied patients was 4.32%. The affected partner was female in 4.93% of cases and male in 2.47% of cases. All of the chromosomal abnormalities observed were translocations: four were reciprocal translocations and three were Robertsonian translocations. In addition 6 patients presented structural variants referred as heteromorphic.
Chromosomal analyses are necessary for establishing the etiology and an appropriate recurrence risk in the couples with recurrent abortions and stillborn malformed child.

J04.02
Carrier woman with a duplication of MECP2 gene - case report

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Mutations in the MECP2 (Methyl-CpG-binding protein 2) gene are responsible for neurodevelopmental disorders like Rett syndrome, mental retardation or severe neonatal encephalopathy. Whole gene duplication along with several other neighbour genes can cause Lubs X-linked mental retardation syndrome (MIM ID #300260) in males. Carrier females may be asymptomatic due to a skewd X inactivation or may have related clinical findings.
We report a case of a woman with mild dysmorphic features as short stature and brachicephaly and negative family history who delivered a Down syndrome afected child, who died few hours after birth. The karyotype performed in order to exclude a chromosomal translocation in the mother, was normal. MLPA testing for the presence of a microdeletion syndrome showed duplication of the MECP2 gene.
Further tests are currently performed in order to better characterize the limits of the genetic defect and to check for its presence in other family members. Prenatal diagnosis is recomanded in a future pregnancy.

J04.03
Evaluation of Abnormal Recombinant Frequency between Chromosomes X and Y in Sperm Nuclei of Patients with Structural Chromosome Aberrations

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Meiosis reduces the chromosome complement by half to generate haploid gametes to allow for genome doubling at fertilization in sexually reproducing organisms. During meiosis, homologous chromosomes undergo pairing, synapsis, recombination, and faithful segregation. In male, sex chromosome recombination behaviors are fundamentally different from female meiosis. Recombination between chromosomes X and Y is normally restricted to the pseudoautosomal regions located at both ends of the sex chromosomes. An obligate crossover in the pseudoautosomal region (PAR1) during male meiosis seems to be necessary for male fertility. Defects in meiosis are a leading cause of both infertility and birth defects (trisomy and monosomy) in humans. In the present study, abnormal recombination frequency between chromosome X and chromosome Y was estimated in sperm nuclei of patients with structural chromosome aberrations such as reciprocal, Robertsonian translocations and other structural changes by using FISH technique. We report herein our results on the incidence of abnormal recombinant products in sperm nuclei as related to structural chromosome aberrations.
This study was supported by Selcuk University Research Fund (No:09102001).

J04.04
Polymorphism of genes and pregnancy loss

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The genetic factors are leading to pregnancy loss, especially in the early stages. It is established that the syndrome of fetal loss is caused not only by chromosomal mutations, but also polymorphism of some genes. These genes can be divided into several functional groups. The purpose of this study was to investigate the polymorphism frequency of some genes (coagulation factors genes and folate cycle protein genes) in women of two groups: a control group and women with the pregnancy loss (8 - 12 week of pregnancy). Placentas were obtained from women with informed consent. The frequencies of gene polymorphisms (C807T ITGA2, -675 5G/4G PAI1, Ala222Val MTHFR, Ile22Met MTRR, Asp919Gly MTR) in control group and among women with pregnancy loss are identical. The combined analysis of genotypes also did not show difference among two groups of women.

J04.05
Polymorphic variants on chromosomes in infertile couples.

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Cytogenetic heteromorphisms are described as heritable variations at specific chromosomal regions without a proven impact on phenotype.Reproductive failure represent a problem for many couples.We have studied the heterochromatic region polymorphism and pericentric inversions in infertile couple (recurrent abortions or primary infertility).
Cytogenetic heteromorphisms are described as heritable variations at specific chromosomal regions without a proven impact on phenotype.Reproductive failure represent a problem for many couples.We have studied the heterochromatic region polymorphism and pericentric inversions in infertile couple (recurrent abortions or primary infertility).
Heteromorphisms of chromosome 9 are among the most common variations in the human karyotype(1-3%). The pericentromeric polymorphisms of chromosome 9 include variations in the size of q-arm heterochromatin, pericentric inversions, and rarely, additional C-band-negative, G-band-positive material. Heteromorphism of heterochromatic regions of chromosome 1,9,16 is also frequent polymorphic variant.
In this study we compared the presence of chromosome heteromorphisms in the karyotypes of two patient groups. The first group of patients consisted of 248 individuals of 124 infertile couples attending an our IVF department with primary infertility or recurrent miscariages. The second group, consisted of 530 amniocentesis samples (for abnormal serum screening). This group was considered to be a sample of the fertile population (spontaneous pregnancy).
Results: 21 individuals (8,46%) and 11 fetuses (2.07%) were found to have chromosome heteromorphisms.
Conclusions: It is suggested that variants should not be ignored by cytogeneticists. Screening prospective gamete donors for chromosome variants may help enhance the success of IVF.

J04.06
Y chromosome evaluation in spontaneous abortion cases

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Introduction: In gestation before the 22nd week of duration, spontaneous abortion (SAB) is the expulsion of an embryo or fetus due to accidental trauma or natural causes. It is one of the most common adverse pregnancy outcomes, affecting up to 15% clinically recognized pregnancies. Etiology of SAB has been tried to be explained by lots of studies however the results are still controversial. In these researches, chromosomal anomalies and Y-micro deletions are considered to be the main reason of SAB, except the advanced maternal age and teratogenic factors. Primarily, cytogenetic evaluation was performed from peripheral blood samples of the couples in spontaneous abortion cases. Additionally, the frequency of Y-micro deletions of these patients and the same control group was examined.
Material-Methods: 30 women who had at least two spontaneous abortions and their husbands were karyotyped to detect the chromosome anomalies. Conventional karyotyping from peripheral blood samples with Giemsa staining was applied. Deletion Detection Kit (Promega- USA) was used for Y-microdeletion analyses.
Results: All the women were 46, XX and the men were 46, XY. No Y-microdeletion was detected in AZFa, AZFb and AZFc regions.
Conclusion: Environmental and genetic factors seem to play combinational roles in the etiology of SAB. In our study we couldn't

detect any causative results in patients. More studies including the teratogenic and genetic factors with high sample number need to be carried out to have an informative knowledge in the etiology of SAB.

P05 Prenatal and perinatal genetics

P05.01
Genetics staffs role at maternity ward

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[Introduction]
As for prenatal service, genetic counseling is basically offered before and after genetic test in Japan. It sometimes makes difficult us to follow up patients continuously. However, at our hospital, genetic staffs have opportunities to see patients at maternity visits and also at the delivery. In this report, we reconfirm our role for the patients and for midwives at maternity ward.
[Case 1] 39 y/o. Para 0 Gravida 0. At 29 weeks, congenital heart defect and single umbilical cord were detected at the growth scan. At 31 weeks, the fetus was diagnosed as 18 trisomy by amniocentesis.
[Case 2] 33 y/o. Para 0 Gravida 0. At 12 weeks, she was referred to our hospital because of NT thickness. After genetic counseling, she declined any prenatal test and had been followed by former clinic. At 27 weeks, she was referred to our hospital again because of polyhydramnios. She decided to have chromosome test at that stage and diagnosed as 18 trisomy.
[Case 3] 36 y/o. Para 1 Gravida 1. At 31 weeks, she was referred to our hospital because of intrauterine growth retardation, omphalocele and congenital heart defect. She declined further prenatal test after genetic counseling. At 35 weeks, we had to remove amniotic fluid because of polyhydramnios, and then she desired to have chromosome test and diagnosed as 18 trisomy.
[Conclusions]
The diagnosis of chromosomal anomaly could be physical and mental stress for patients. We consider that the continuous support both genetics staffs and maternity ward is very important.

P05.02
Evidence of unique mutation in Maghrebin Allgrove's patients allows rapid molecular diagnosis and easy genetic counseling

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Allgrove syndrome or Triple A syndrome is an autosomal recessive disease including a clinical triad; Alacrima, Achalasia, and Addisonism. Progressive neurological impairment could be associated.
The defective gene AAAS, located in 12q13, encodes a WD-repeat protein, ALADIN. Tullio pellet suggested that this disease in Maghreb is associated to an ancestral founder mutation, the same in six Algerian and three Tunisian families).
The classical triad was not required to enclose patients in our study. Alacrima associated to achalasia and/or adrenocortical insufficiency was sufficient criteria. In our study, we include twenty two patients belonging to fourteen Maghrebin families, twelve from Tunisia and two from Libya. The molecular analysis targets the major mutation in AAAS gene. In our study, we used the (PCR-RFLP) assay with sequencing method to valid the mutation IVS14 + 1G → A.
Clinical analysis showed variable expressivity but constant alacrima and also consanguinity in all families. The molecular analysis showed that all patients were homozygote and all parents were heterozygote for the same mutation (IVS14 + 1G → A).
The (PCR-RFLP) assay is an easy technique to detect and descry healthy heterozygote, healthy homozygote and affected homozygote. The sequencing method is used to check and valid the mutation in each family.
As the mutation in maghrebin Allgrove's patients seems to be unique and the PCR-restriction fragment length polymorphism assay allows a rapid molecular diagnosis, the genetic counselling became easier. Neonatal molecular diagnosis allows an early optimized follow-up, while prenatal diagnosis raises ethical queries'.

P05.03
A case of mosaic karyotype 45, XO/46, XY - [75%]/ [25%] detected by QF-PCR and confirmed with cytogenetic analysis

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Rapid diagnosis by Quantitative Fluorescent PCR analysis (QF-PCR) has proved its cost-efficiency, speed and efficacy for detection of the most common autosome aneuploidies - trisomy 21, trisomy 18, trisomy 13 and some of sex chromosomes aneuploidies. Besides of its wide application in prenatal diagnosis, QF-PCR is very useful for rapid sex determination of neonates when needed.
We report a case of mosaic karyotype 45, XO/46, XY - [75%]/ [25%] detected by QF-PCR and confirmed with cytogenetic analysis. A neonate, born of second normal pregnancy of young healthy parents was referred to the National Genetic Laboratory for genetic counseling and sex determination. The baby had ambiguous genitalia (severe hypospadic micropenis and bilateral crytorchism) and mild facial dismorphism.
QF-PCR and cytogenetic analysis were performed on peripheral blood. DNA was amplified with commercial QF-PCR kit Aneufast (Molgentix SL, Barcelona, Spain) and analyzed on ABI 3110 XL. Cytogenetic analysis was performed on G-banded metaphases from cultured lymphocytes at resolution 400 bands.
QF-PCR showed results consistent with an aneuploidy, SRY was present. Pseudoautosomal marker AMEL (Xp22.31-22.32/ Yp11) showed a ratio 4:1. Markers on chromosome X - HPRT, DXS6803, SMBA, DXS6809, DXS8377 were in hemizygous pattern. A week later cytogenetic analysis revealed 45, XO/46, XY - [75%]/ [25%] karyotype. Our 10-years experience shows that DNA analysis is informative in cases of mosaics but cytogenetic analysis should be held for a complete diagnosis.

P05.04
Detection of hard palate abnormalities using 3D ultrasound

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Objective: The aim of this study was to asess the three-dimensional ultrasound technique in prenatal detection of hard palate abnormalities.
Methods: 82 cases of suspected cleft palate, diagnosed by routine 2D ultrasound scan, were referred to us for a detailed assesment of the lips and palate, using 3D ultrasound. A G.E.Voluson 730 Expert was used by two experienced operators,one of them scaned using 3D reverse-face view technique,and the other scaned using angled insonation and anterior axial techniques,all of which seek to maximize visualizationof the palate without shadowing.
Results: Of 82 pacients, in 8, 3D views were not obtained, leaving 74 cases for asessment. The sensitivity for the diagnosis was 86,3%, false positive rate was 12% and false negative rate was 9%.
Conclusion: The data reported suggest that 3D ultrasound is a very usefull method of prenatal evaluation of the fetus, and can give parents a more detalied asessment of the nature and extent of facial and hard palate clefts; the most important advantage of 3D volume scanning is the ease and rapidity with which the different planes can be accessed.

P05.05
Clinical use of array comparative genomic hybridization (aCGH) for prenatal diagnosis in 1000 cases

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Array-based comparative genomic hybridization (aCGH) allows for fast and accurate detection of chromosomal aneuploidies, submicroscopic deletions or duplications and other unbalanced chromosomal abnormalities. While experience with diagnostic aCGH in the genetic evaluation of pediatric patients is extensive, experience with its use for clinical prenatal diagnosis is still limited.

To assess the feasibility of offering CGH microarray for prenatal diagnosis as a first-line test, a blind study was conducted comparing the results obtained from array CGH with those obtained from a standard karyotype.

Women undergoing amniocentesis or chorionic villus sampling (CVS) for karyotype were offered aCGH analysis using whole-genome BAC arrays with an average resolution of 0.5-1 Mb. A total of 1000 prenatal samples were processed in parallel using both aCGH, performed on DNA isolated from uncultured AF (83%) or CVS (17%), and G-banded for standard karyotyping.

Chromosomal abnormalities were identified in 34 (3.4%) samples; in 10 of which (29.4%) aCGH detected pathogenic copy number variations (CNVs) that would not have been found if only a standard karyotype had been performed.This study demonstrates that aCGH represents an improved diagnostic tool for prenatal detection of chromosomal abnormalities. Although larger studies are needed, the above results provide a further evidence on the feasibility of using aCGH as first-line diagnostic test to detect chromosomal abnormalities in prenatal samples.

P05.06
Preimplantation Genetic Diagnosis (PGD) for Cartilage Hair Hypoplasia combined with Human Leukocyte Antigen (HLA) matching

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Cartilage-hair hypoplasia (CHH) is a rare autosomal recessive disorder, characterised by dwarfism, hypoplastic hair and immunodeficiency. Allogeneic hematopoietic stem cell transplantation (HSCT) has been shown to be an effective treatment for CHH patients. We present a couple, both carriers of different ribonuclease mitochondrial RNA-processing (RMRP) mutations underlying CHH, which relied on In Vitro Fertilisation (IVF) and PGD to select unaffected embryos that were HLA compatible with an affected compound heterozygous sibling.

Preclinical workup involved confirmation of the maternal g.126C>T and paternal g.40G>A mutations in genomic DNA of the couple and affected child, together with informativity and segregation testing of RMRP polymorphic markers (STR16GT at 248 kb 5' and STR16AAT at 34 kb 3') and markers in the HLA locus of 4 Mb (MOG3, D6S2700, D6S2670, D6S2443, D6S1560 and D6S1618). (Half)-informative HLA and RMRP markers were selected and combined with the amplicon of the mutations in a single-cell multiplex PCR protocol. The mutations were detected using post-PCR minisequencing reactions. The RMRP markers ensured an accurate diagnosis of the mutations whereas the HLA markers provided indirect preimplantation HLA haplotyping.

The couple achieved an ongoing singleton pregnancy in their third IVF/PGD cycle and the PGD results were confirmed in prenatal testing. At birth, hematopoietic stem cells will be collected from the cord blood and used to transplant the affected sibling.

This report shows that PGD with HLA matching is an alternative therapeutic option for couples of reproductive age with children affected by CHH requiring a non-urgent HSCT and lacking suitable donors.

P05.07
Multicenter validation of reverse-hybridization teststrips for the detection of common CYP21A2 mutations in dried blood spots.

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Congenital adrenal hyperplasia (CAH) comprises a group of inborn errors in the synthesis of adrenal corticoid hormones. More than 90% of cases arise from mutations in the CYP21A2 gene encoding steroid 21-hydroxylase. The average incidence of classical CAH is about 1 in 15,000 births worldwide. Newborn screening programs based on 17-hydroxyprogesterone (17-OHP) levels have been introduced in various countries, but due to the considerable false positive recall rate a concurrent genetic testing would be desireable.

We have developed a reverse-hybridization teststrip-based assay for the rapid and simultaneous analysis of the following 11 common CYP21A2 mutations from dried blood spots: P30L, I2 splice (655A/C>G), Del 8bp E3, I172N, I236N/V237E/M239K (Cluster E6), V281L, L307Frameshift, Q318Stop, R356W, P453S and R483P. Automated instrumentation and use of a scanner-based software tool for recording and interpreting results can make the StripAssay a useful tool in CAH newborn screening programs.

The new CAH StripAssay was validated in a series of 243 DNA samples of known CYP21A2 genotype. By using the StripAssay in combination with a real-time quantitative PCR approach (Parajes et al., 2007), all 11 mutations covered plus CYP21A2 copy number variations and chimeric genes were unambiguously identified. Our results have been confirmed by DNA sequencing and MLPA. In 19 (7.8%) probands of our heterogeneous European cohort we found at least one rare CYP21A2 mutation that was not represented on the teststrip; however, as several of these represented members of the same family the prevalence of rare mutations is expected to be lower. (oberkanins@viennalab.co.at)

P05.08
Array-CGH in fetuses with ultrasound anomalies and normal karyotype

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Background: congenital malformations are the main cause of infant morbidity and mortality during the first year of life. Approximately 18-30% of fetuses with congenital malformations have chromosomal imbalances. However the causes of defects remain unknown in almost 50% of cases.

Methods: a retrospective study in 40 fetuses with isolated or multiple malformations , and/ or Intrauterine Growth Restriction detected by ultrasound, were done using a 8x60K Agilent-based custom oligo genome-wide array(**KaryoArray®**). The average density of the probe coverage is 43 kb, comprising specific probes covering microdeletion and microduplication syndromes, telomeres and peri-centromeric regions, including more than 350 clinically relevant regions.

Samples were from CVS, AF, fetal blood and tissues (fibroblasts and others). Parents' studies by array-CGH were done when genomic imbalances were found.

Results: 6/40 genomic imbalances with pathological significant were identified: one Complex Chromosomal Rearrangement (CCR), one 8p terminal deletion resulting from malsegregation of a criptic maternal balance translocation, two cases of mosaicism (1q duplication and a trisomy 8, both detected in tissues), one 9q interstitial deletion, and one 17q12

microdeletion syndrome (RCAD: renal cyst and diabetes). Additionally several polymorphic variants were detected, some of them inherited.
Conclusions: array-CGH improved overall detection rates of clinically significant chromosomal abnormalities in fetuses with sonographic anomalies and normal karyotype. The use of this technology will have an important impact on genetic counseling in pregnancies with fetal anomalies. The possibility of having an accurate genetic diagnosis will decrease the anxiety of couples facing a future pregnant.
This study was granted by FIS-08/1207

P05.09
DFNB1 markers associated with nonsyndromic hearing loss in Romanian children

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Background: The DFNB1 locus has been linked to a nonsyndromic “invisible handicap” called congenital sensorineural hearing loss (HL) and deafness. Mutations of the GJB2 and GJB6 genes are both associated with deafness at the DFNB1 locus. The diagnosis of DFNB1 is made with molecular genetic testing.
Here we report the first study of D13S1830 and D13S1854 markers performed in Romania.
Objective: Our goal was to use molecular testing of children at risk for identifying the contribution of the DFNB1 markers to prelingual HL; these markers are known to be of high prevalence in other European populations.
Patients and Methods: This study was carried out on 253 children with prelingual HL, originating from various regions of Romania. Blood samples were obtained and DNA was isolated by standard protocols. The 35delG GJB2 mutation was detected by ARMS-PCR. We included beta-actin sequence detection as internal control. Analysis of del(GJB6-D13S1830) and del(GJB6-D13S1854) was performed by multiplex-PCR.
Results: GJB2 mutations were detected in 33% of patients, all of them having 35delG homozygous mutation. Only 3% of children presented 35delG mutation in a heterozygous form. None of them was found to have the GJB6 mutations. Physical examination revealed no other abnormal findings.
Conclusions: The identification of the most frequent causal mutation of prelingual HL in Romanian population will contribute to develop both prenatal and postnatal molecular diagnosis protocols well suited for our population.

P05.10
Residual risk of other chromosome abnormalities following NIPD for Down Syndrome

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Background:Non-invasive prenatal diagnosis (NIPD) using next generation sequencing is impending. Reports to date suggest that initially NIPD will test for Down Syndrome (DS) only. One essential piece of information that needs to be considered will be women’s residual risk for the chromosome abnormalities that NIPD won’t detect.
Aim: To calculate the residual risk of fetal chromosome abnormalities in women at increased risk of DS if they had NIPD for (1) DS only (2) DS, plus Trisomy 18 and Trisomy 13 (3) DS, plus Trisomy 18, Trisomy 13, and sex chromosome aneuploidies.
Method: A population based data set of all prenatal diagnostic tests in Victoria, Australia, for 2008 (70,000 births) was used. Frequencies of abnormal fetal karyotypes amongst women referred for an increased risk of DS were extracted.
Results: There were 2954 amniocenteses or CVS for increased risk of DS, and 287 chromosome abnormalities detected (1 in 10). Of these, 129 were DS i.e. 1 in 23 overall.
This means that 1 in 19 pregnancies had some other chromosome abnormality (residual risk). Inclusion of T18 and T13 in the detection panel reduces residual risk to 1 in 30, and if sex aneuploidies are added, to 1 in 42.
Conclusion: In a population of women at increased risk of DS, the risk of non-DS chromosome abnormalities is higher than the risk of DS. Women need to be informed about the spectrum of chromosome disorders currently detected by karyotyping that will no longer be detected by NIPD for DS.

P05.11
The effectiveness of prenatal cytogenetic diagnosis of Down syndrome in the Republic of Moldova.

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Prevention of chromosomal pathology and, in particular, Down syndrome (DS) is the main task of the medical genetic services. The average frequency of trisomy 21 or SD in the EUROCAT is 9,5 / 10 000 births.
The aim of the study was to evaluate the effectiveness of prenatal cytogenetic diagnosis of DS in Moldova.
To obtain the epidemiological characteristics of Down syndrome were used monitoring data DS for the period from 2005 to 2009; the total number of births during this period was 193076. DS was diagnosed in 209 children. DS in fetus were carried out in 16-18 weeks of pregnancy by amniocentesis and cytogenetic diagnosis in 232 women, 23 pregnancies were terminated.
The indications for karyotyping were: age 35 and older, the presence of ultrasound markers of fetal chromosomal pathology, family history, balanced chromosomal translocations in relatives, the deviations of maternal serum markers and the combination of these factors.
The average frequency of DS in Moldova in 2005-2009 was 12.02 / 10 000 (1:924). In 21% of DS cases the age of mothers was 35 years and older, as well as age younger than 18 years. The frequency of DS in 2005-2009 in the population of the Republic of Moldova was 1 for 924 births. Excluding the data of prenatal cytogenetic diagnosis of DS in the frequency of Moldova would be 1:832 newborns. The effectiveness of prenatal cytogenetic diagnosis of DS during II trimester of pregnancy for the analyzed period was 10%.

P05.12
Application of multiplex real-time PCR assay using TaqMan MGB probes on CVS and amniocyte samples for prenatal diagnosis of trisomy 21

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DOWN syndrome (DS), the most common fetal aneuploidy, is caused by an extra copy of chromosome 21 (trisomy 21) affecting 1 in 700-1000 live births Therefore diagnosis and prevention of live-born affected fetus is a health care priority.
We have successfully used a novel MGB TaqMan probe-based real time PCR assay for rapid diagnosis of trisomy 21.
Chorionic Villus Samples (CVS) and **amniocyte samples** was obtained from 59 pregnant women who had the high risk criteria of having fetus with trisomy 21. Quality and quantity of DNA was measured by the optical absorbance. DYRK1A and DSCAM genes (target genes) and PMP22 (reference gene) were selected and specific primers and probs for these genes and were designed. After determining of standard curve and PCR efficiencies for each gene, the target/reference genes ratio was calculated using comparative cycle threshold method .
The results of gene dosage analysis showed the target/reference genes ratio of 1.56±0.09 and 1.02±0.11 in trisomy 21 and normal samples for Chorionic Villus Samples and 1.61 ± 0.09 and 1.03 ± 0.05 for **amniocyte samples** respectively that showed statistically significant difference between two groups.
The conventional cytogenetic analysis needs live cultured cells and is too time-consuming for clinical application. In contrast, molecular methods such as FISH, QF-PCR, MLPA and quantitative Real-time PCR are rapid assays with results in 24h.Therefore, Real-Time PCR technique can be used as an accurate, rapid and sensitive method for prenatal diagnosis of trisomy 21.

P05.13
A novel digital PCR platform accurately detects and quantifies fetal DNA in maternal plasma

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Determination of fetal load in maternal plasma is a pre-requisite for a number of non-invasive approaches to detecting fetal abnormalities. Existing techniques are faced with difficulties when trying to quantify the minute amounts of fetal DNA in maternal plasma, particularly for female fetuses. A new droplet digital™ PCR (ddPCR™) system was developed to address these challenges.

The ddPCR system partitions the sample into 20,000 droplets, each one nanoliter in volume, such that some contain the target of interest and some do not. The emulsion is thermocycled to end-point, then every droplet is read with a two-color fluorescence detector. The number of positive and negative droplets is used to compute an absolute concentration of the target with high precision and accuracy. The simple workflow uses standard 96-well plate processing enabling the system to generate data for millions of PCR replicates within a matter of hours.

The assay uses a SRY Y-chromosome marker for male fetuses and methyl-sensitive restriction enzyme digestion of a RASSF1A marker as a gender-independent test. We applied this method to the analysis of more than one hundred maternal plasma samples to obtain a distribution of total DNA and fetal load.

P05.14
Different application of Y-specific sequence detection using cell free fetal DNA from maternal plasma

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Noninvasive prenatal identification of fetal gender is currently feasible through analysis of cell free fetal DNA from maternal plasma. Two approaches to fetal sex prediction, testing *DYS14* and *SRY* sequence, were compared with respect to precision, sensitivity, specificity and reproducibility of the test. Quantification of the fetal single-copy *SRY* sequence was evaluated as a precondition for the investigation of quantitative abnormalities in cell free fetal DNA concentration for pregnancy-associated pathologic conditions such as preeclampsia or intrauterine growth retardation.

Thirty-seven plasma samples from pregnant women taken between the 15th and 18th week of gestation were analysed. Real-time PCR analysis employing TaqMan technology was used for amplification of fetal Y-specific sequences *DYS14* and *SRY*. A blind test for final validation used anonymized plasma samples, and the fetal genders of all analyzed samples were confirmed after birth.

The results confirmed that both protocols are reliable methods for fetal gender determination, with sensitivity, specificity and accuracy rates of 100% having been observed for both approaches. Higher reproducibility with a lower coefficient of variation for multi-copy *DYS14* protocol was observed in comparison with *SRY*. A distinct shift of nearly 4 Ct values was detected between both protocols. The detection limit for *SRY* assay reached 3 GE/ml and the mean concentration value for single-copy *SRY* sequence was 15 GE/ml .

Our study confirmed *DYS14* protocol as a precise method for assessment of fetal gender whereas *SRY* assay as an appropriate precondition for monitoring of pathologic states in pregnancy.

P05.15
Amniocentesis after established carrier status of balanced structural chromosome abnormality

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About 6% of couples investigated in our laboratory for recurrent miscarriage are carriers of a structural chromosome abnormality. We offered all of them prenatal diagnosis for future pregnancies. We collected data from couples with chromosomal rearrangement investigated for karyotyping after two or more miscarriages. Twentyfour couples were identified with chromosomal inversions and 13 of them had prenatal diagnosis performed, as following: 1 case with inv(7), 1 case with inv(10) and 11 cases with inv(9). In all cases amniocentesis showed fetuses carriers of invesions and pregnancies continued. We encountered 7 couples with Robertsonian translocations: 4 cases with trob(13;22) and 3 cases with trob(13;14). At one case of each category, prenatal diagnosis was performed and pregnancy evolved normaly. One of these fetuses was normal and one was a carrier with balanced translocation trob(13;22), just like the mother. We found 26 couples with a balanced translocations. Seven of these couples performed prenatal diagnosis. Parental karyotype showed the following translocations: 46,XY,t(1;5)(q23;p12), 46,XX,t(3;15)(q12;q13), 46,XX,t(4;10)(q22.2;q22.2-ter), 46,XX,t(7;9)(p14q24), 46,XX,t(7;10)(p22;p12.1), 46,XX,t(17;20)(q12;q11), 46,XY,t(15p;19p). Fetal karyotype showed 3 normal fetuses, 4 carriers of a balanced translocation and one carrier of non-balanced translocation 46,XY,dup(10q22.2-ter), consequently this last pregnancy was terminated. One couple performed twice amniocentesis and fetal karyotype showed in both cases carriers of the balanced translocation t(7;10)(p22;p12.1), as was their mother. Detection of a structural chromosome abnormality in couples with recurrent miscarriage has a striking influence on their decision to undergo prenatal diagnosis. Genetic counseling helps these couples to understand the recurrence risk and consequently to evaluate their options.

P05.16
Improvement of the recovery of fetal nucleated red blood cells (NRBCs) and its application to the prenatal genetic diagnosis.

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Fetal nucleated red blood cells (NRBCs) have emerged as the best candidates for noninvasive prenatal diagnosis. But fetal NRBCs are rare in the maternal circulation. To assess the detection efficiency of the isolation of fetal NRBCs using the different density gradients and different osmolality of percoll, we counted number of NRBCs in the blood of pregnant women at different gestational ages.

Fetal NRBCs were isolated from 10ml of maternal in 179 cases using double percoll gradient with optimal osmolality (280 mOs/kg$H_2$0, 1.077 g/ml and 520 mOs/kg$H_2$0, 1.119 g/ml). Magnetic activated cell sorting was used to enrich isolated NRBCs, and morphological differentiation was performed with Kleihauer-Betke stain. Fluorescence in situ hybridization (FISH) analysis was used to detect of chromosomes 13, 18, 21, X and Y.

The mean number of NRBCs were 8.73 (6 to 14 week of gestation), 15.24 (15 to 20 week of gestation), 17.52 (21 to 28 week of gestation) and significantly increased during the period from 6 to 28 weeks of pregnancy (p=0.043, p=0.036). The frequencies of fetal NRBCs were 71.35% (6 to 14 week of gestation), 60.78% (15 to 20 week of gestation), 55.83% (21 to 28 week of gestation). There was a significant increase in the frequency of fetal NRBCs in a first trimester compared to a second and third trimester (p=0.005).

The NRBC enrichment method using optimal osmolality in first trimester can be a more useful prenatal diagnostic tool for fetal aneuploidy than in second trimester.This research was supported by grant SC1150 .

P05.17
Detection of genetic abnormalities by array comparative genomic hybridization for fetal tissues

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Introduction: Array-CGH is a microarray-based technology which can be used to perfoming molecular karyotyping. The use of array CGH in genetic diagnosis has originated a new perspective in methodology of

fetal tissue diagnosis. Whole genome analysis with high resolution can be performed efficiently through this technology. Our aim is to detect genetic aberations in fetal tissues by array CGH method.

Materials And Method: Materials included nine fetal tissues which are from terminated pregnancies that are diagnosed in Gynecology and Pathology departments of Kocaeli Medical Faculty. Whole genome analysis was performed in Medical Genetic Department of Kocaeli Medical Faculty. gDNAs of patients were hybridized with array CGH platforms using syndrome Plus ISCA Microarray 4X44K format (Oxford Gene Technology Oxford, UK). Hybridized microchips were scanned with Agilent Microarray scanner. The data were pooled in Agilent Feature Extraction software and normalized using CytoSure Analysis Software v.2.0.8

Results: We found dup(7)(q31.1-q36.3) and del(18)(p11.23-11.32) in fetal tissues. We confirmed the abnormalities of fetal tissues with parental chromosome analysis. Maternal karyotype was t(8;17)(q31.1)(p11.2). This balanced translocation carrier mother had imbalanced fetus with partial trisomy and partial monosomy.

Conclusion: Our findings indicate that array CGH has been a useful tool in discovering microscobic and submicroscobic genetic changes in fetal tissues.

P05.18
FGFR-3 Related Skeletal Dysplasias Diagnosed Prenatally by Ultrasonography and Molecular Analysis: Presentation of 17 Cases

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Fibroblast Growth Factor Receptor-3 (FGFR-3) related skeletal dysplasias are caused by mutations in the FGFR-3 gene that result in increased activation of the receptors alterations in the process of endochondral ossification in all long bones, and include achondroplasia, hypochondroplasia, thanatophoric dysplasia and severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN). Reports of prenatal diagnosis of FGFR-3 related skeletal dysplasias are not rare, however, the correlation between 2nd trimester ultrasonographic (U/S) findings and underlying molecular defect in these cases is relatively poor. There is a need for specific U/S predictors than can distinguish lethal from non-lethal cases and aid an earlier prenatal diagnosis. Here we present sixteen (16) sporadic and one (1) familial cases with FGFR-3 related skeletal dysplasia and a normal karyotype. We evaluate biometric parameters and U/S findings consistent with the molecular diagnosis of skeletal dysplasia. U/S scan performed even at the 18th week of gestation can indicate a decreased rate of development of the femora (femur length<5th centile), while the mean gestational age at diagnosis is still around the 26th week. The utility of other biometric parameters and ratios is discussed (foot length, BPD, HC, FL/foot and FL/AC). In two cases of discontinued pregnancy, fetal autopsy led to a phenotypic diagnosis and confirmed the prenatal prediction of lethality. We conclude that the combination of U/S and molecular genetic approach is helpful for establishing an accurate diagnosis of FGFR-3 related skeletal dysplasias *in utero* and subsequently for appropriate genetic counselling and perinatal management.

P05.19
Facilitating the Fragile X post- and prenatal genetic diagnostic testing workflow by use of the Abbott FMR1 TP-PCR and FMR1 sizing PCR products.

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Molecular testing of FMR1 (CGG)n expanded repeats remains hard to tackle given the limitations of sizing PCR in detecting large and uninformative alleles, resulting in a significant number of samples that require confirmation through laborious southern blot analysis. Abbott Molecular recently developed a TP-PCR to be used in combination with their sizing PCR to facilitate the Fragile X diagnostic testing workflow.

This study aimed at comparing Abbott Molecular FMR1 Sizing PCR and TP-PCR versus in-house sizing PCR and southern blot in order to evaluate the sizing accuracy and detection sensitivity of normal, intermediate, premutation and full mutation alleles. Over 100 samples of different sources (approximately 50% whole blood, 40% chorion villi and 10% amniocytes) and a panel of artificially mimicked mosaic samples were evaluated.

Signal intensity and sizing accuracy met expectations in the normal to small premutation range. The sizing 'long run' greatly improved the detection capacity and sizing accuracy of longer range (large premutation to full mutation) fragments, although inspection of raw data is recommended. TP-PCR allowed discrimination between normal/intermediate and premutation/full mutation alleles. However, premutation and full mutation TP-PCR signal patterns were very similar, therefore requiring the Abbott FMR1 Sizing PCR to distinguish them.

Mosaic detection in Sizing- or TP-PCR ranged from 20% down to 10%, depending on the repeat range mixture used.

Our results corroborate the workflow proposed by Abbott Molecular of using FMR1 TP-PCR as a first line screening platform in combination with the FMR1 Sizing PCR as second line test.

P05.20
Evaluation of a CGG Repeat Primed PCR system, designed for detection of Fragile X expanded alleles, in clinical prenatal samples.

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Abnormal expansion of the $(CGG)_n$ repeat in the 5'untranslated region (UTR) of the *FMR1* gene is known to generate Fragile X syndrome (FXS), the most common form of cognitive impairment, and several other diseases in patients. The expansion reduces the expression of the *FMR1* by promoting DNA hypermethylation of this region when exceeding ≈ 200 repeats. Molecular diagnosis relies on determination of the number of allele repeats in the DNA template, and assessment of the methylation state of the *FMR1* locus. Current conventional PCR amplification is only successful for normal and small permutation alleles, and is not informative for homozygous repeat alleles. Expanded alleles too large to amplify efficiently and female homozygous samples require additional Southern Blot (SB) analysis for categorization and sizing. Although regarded as gold standard, this technique is laborious, time consuming and involves (not always available) large amounts of DNA. However, accurate and efficient quantification of the number of repeats in the 5' UTR of *FMR1* gene is essential as premutation alleles are common in the general population.

We evaluated a commercially available assay (Asuragen) using a large set of blinded archived prenatal samples that were previously analyzed for FXS with conventional PCR and SB analysis in our Center. The PCR reagentia were able to identify accurately and quickly *FMR1* genotypes, ranging from normal over premutation to full mutation alleles, in patient and control samples. Exact sizing was possible for a spectrum of permutation alleles. The assay is also sensitive to size mosaicism and AGG interruptions.

P05.21
Isolation of fetal free nucleic acids from maternal plasma and detection of fetal gender

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The detection of fetal cells and free fetal DNA opened new horizonts in the non-invasive prenatal diagnosis. We aimed to try out two types of isolation kits and to compare the effect of the quantity of the maternal plasma ont he concentration of the obtained DNA.
We collected EDTA blood samples from 40 pregnant women. Samples were centrifuged and the supernatants were stored on -85 ºC until use. We used two kits for DNA isolation from the maternal plasmas, High Pure PCR Template Preparation kit (Roche, Germany) and NucleoSpin Plasma XS kit (Macharey Nagel GmbH, Germany). In the case of the NucleoSpin we increased the quantity of the initial plasma to get correct comparison. DNA concentrations were measured by Qubit Fluorometer (Invitrogen, USA). SRY primers and probes were used by quantitative real-time PCR for determination of fetal gender.
The concentration of the free DNA were almost the same by using equal quantity of the initial plasma (2.96±1.04 ng/ml and 3.09±1.76 ng/ml), while it was significantly lower when we used smaller quantity as recommended with Nucleospin. We were able to detect the fetal sex in all samples correctly.
According to our results it seems that the concentration of the isolated free nucleic acids depends on the initial maternal serum quantity.

P05.22
Relationship of high pregnancy associated plasma protein-A (PAPP-A) levels to demographics and pregnancy outcomes

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There are many studies on the adverse effects of low pregnancy associated plasma protein-A (PAPP-A), but very few studies on high PAPP-A levels which creates uncertainty and stress on pregnancy counselling.
We retrospectively analyzed the PAPP-A levels and demographics of 12,428 pregnant women who were seen at Yale University Hospital prenatal unit between November 2002 and November 2008. Among these 12,428 pregnancies we first investigated the PAPP-A above 5 MoM, then the values between 4 and 5 MoM. For both groups we excluded the twin pregnancies and other invalid data.Then we compared the results with the general population. (It is estimated that 2.5% of newborns have a recognizable malformation at birth.)
There were 47 pregnancies (0.38%) with PAPP-A above 5 MoM. After exclusion, 23 pregnancies remained. 4 pregnancies (17.3%) ended with complications which is too few to draw a statistical conclusion.
There were 78 pregnancies (0.64%) with PAPP-A between 4 and 5 MoM.After exclusion, 72 pregnancies remained. 8 pregnancies (11.1%) ended with complications.This is statistically significant. (p< 0.01).However, we need more studies with bigger population numbers to suppose that the outcome of pregnancy will differ with high PAPP-A values. Our study also provides useful data for examining the relationship between certain demographic factors, such as race and diabetes, and PAPP-A levels. Whites have higher PAPP-A (mean=1.19) than blacks (mean=1.49). (p < 0.001) Diabetics have lower PAPP-A(mean=1.04) than non diabetics (1.22). (p= 0.001)

P05.23
When fate hits a family...!

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We report on a family with hypochondroplasia, in whom the pregnant mother was referred for a detailed ultrasound examination at 33 weeks because of the presence of polyhydramnios and shortening of bones.
Prior to this pregnancy, the diagnosis of hypochondroplasia was established in the daughter of the proband. Molecular studies showed a p.N540K (c.1659C>G) mutation in the *FGFR3* gene. Subsequent investigations revealed that the mother also had this mutation, as well as her son.
The detailed fetal ultrasound of the ongoing pregnancy evoked the diagnosis of achondroplasia rather than hypochondroplasia. Initial molecular testing on amniotic cells failed to detect the p.N540K mutation. Because of the suspicion on fetal ultrasounds of achondroplasia further molecular analysis was performed, and revealed the presence of the classic achondroplasia mutation p.G380R (c1138G>C), hereby confirming the diagnosis of achondroplasia in this foetus.
In conclusion: this baby with a prenatal diagnosis of achondroplasia, was born in a family with a mother and 2 sibs affected with hypochondroplasia. The age of the father at conception was 33 years. Since this is an unusual finding, further molecular studies to determine the parental origin of the p.G380R mutation using flanking polymorphisms, are performed and the results will be discussed. If the mutation is located on the maternal chromosome there might be a mechanism that can explain that the *FGFR3* gene in the mother is more prone to mutations. If the achondroplasia mutation is located on the paternal allele, this remarkable situation is just coincidence.

P05.24
Low Apgar scores linked with idiopathic congenital cataract: a long-term follow-up study in a Romanian primary care centre

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Background: Several studies have shown that low Apgar scores bellow 7 can be linked to different neurologic diseases, but few were related to ophthalmologic disorders. Congenital (or infantile) cataract is a frequent cause of blindness among children.
Methods: In a ten years longitudinal study, between 1 January 1998 and 31 December 2008, we prospectively enrolled and followed up a population-based cohort study of 550 live newborns in a Romanian primary care setting by using medical data obtained after delivery. The Apgar scores at 1 or 5 minutes were recorded following standardized procedures. We obtained information on idiopathic congenital cataract by linking the cohort with the Pediatric Ophthalmology Hospital Register. Cohort members were followed from birth until onset of the ophthalmologic disease or 31 December 2008, whichever came first.
Results: The decreasing Apgar scores were linked with significant increased rate of idiopathic congenital cataract. The incidence rate of idiopathic congenital cataract was 235 per 100,000 person-years for those with 5-minute Apgar scores of 1 to 3 and 75 per 100,000 person-years for those with a score of 10. This incidence remained particularly high in early childhood. Bilateral isolated cataract cases were male dominated (68%), and associated with low birth weight (<2000 g). No significant associations were found with older age (≥40 years) of mothers at delivery or cesarean section.
Conclusions: Neonates with a suboptimal Apgar score have a higher risk of idiopathic congenital cataract that lasts into early childhood.

P05.25
Relatively high frequency of recurrent microdeletion syndromes and subtelomeric deletions/duplications uncovered through non-selective application of a MLPA based extended prenatal panel in routine prenatal diagnosis

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Numerous recurrent genomic disorders have been described, involving gain or loss of genetic material at a level not detectable by routine karyotype analysis, and are the underlying cause of various congenital abnormalities with or without mental retardation. Although individually rare, these genetic syndromes may occur collectively in more than 1 in 1,200 live births, while prenatal ultrasound examination and karyotype analysis will typically fail to detect most, if not all, of the above anomalies in the fetus. Since 2006 we have applied a multiplex ligation-dependent probe amplification (MLPA) based screening approach in 1722 typical unselected prenatal cases, with (16.1%) or without (77.7%) ultrasound findings, for the simultaneous

targeted detection of 23 recurrent microdeletion syndromes as well as subtelomeric copy number assessment for all chromosomes. Following amniocentesis or chorionic villus sampling, samples were processed for routine karyotype analysis while DNA was extracted in parallel for MLPA analysis. When necessary, parental samples were analyzed to determine the inheritance of the detected aberrations. We identified nine (9) fetuses with pathological genomic abnormalities (approximately 1 in 191), five of which had as sole indication advanced maternal age (1 in 240). In 2 cases an abnormality was suspected from karyotype analysis, while the remaining 7 cases would have otherwise remained totally undetected. Our data represent the largest published series involving this type of genomic analysis in routine prenatal diagnosis, without indication bias. The panel increases significantly the diagnostic yield of conventional prenatal chromosomal diagnosis and does not pose major interpretation problems. Furthermore, our findings raise some interesting points to consider and perhaps contribute towards a careful reevaluation of traditional risk assessment and our strategy position, in terms of what we aim for in prenatal chromosomal diagnosis.

P05.26
Targeted inhibitory PCR, an attractive laboratory assay for quantitative detection of fetal DNA isolated from maternal plasma.

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Several methods have been developed for non-invasive prenatal diagnosis (NIPD) based on circulating cell-free fetal DNA isolated from maternal plasma. In particular massive parallel shotgun sequencing (MPSS) is capable to identify fetal trisomies. The accuracy of this approach is dependent on the number of fetal DNA molecules present and the overall fetal fraction. A control reaction that is able to assess the fetal contribution to the DNA sample is therefore highly beneficial.
We have previously developed a multiplex assay that utilized DNA methylation as a basis for discrimination of fetal derived DNA from maternal DNA. First, methylation-sensitive restriction is used to isolate the methylated fetal DNA markers and amplified using competitive PCR in one multiplex reaction. Finally the amplicons are quantified using MALDI-TOF mass spectrometry. This setup allowed us to obtain both fetal and total copy numbers and the overall fetal fraction.
To minimize the complexity of the MPSS workflow, we have modified this fetal quantifier assay to integrate with standard laboratory equipment already used in the MPSS process. We have developed a novel PCR strategy where the amplification of the abundant total DNA markers is modulated using target specific PCR primer inhibitors. Finally the amplicons are quantified using standard electrophoresis instrumentation. Using this setup we have screened 200 DNA samples obtained from pregnant women and non-pregnant women. Our results show that this rapid and high-throughput assay can reliably detect and quantify the small fraction of fetal DNA isolated in maternal plasma.

P05.27
A validation study for non-invasive fetal sexing and the comparison of different maternal discrimination tests in diagnostics.

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While prenatal diagnosis for fetal sex determination mainly relies on invasive procedures which carry a risk of fetal loss, there is a growing need to explore more alternative tests for non-invasive prenatal diagnosis early in gestation.
We have explored the application of free fetal DNA (ffDNA) from maternal plasma for non-invasive fetal sexing and maternal discrimination tests.
In 2008, we started a large validation and implementation study for non-invasive fetal sexing from maternal plasma in diagnostics. In this study 209 pregnant women were included (n=112 male and n=97 female pregnancies).
As described earlier we used a combination of Real-Time PCR and Pyrophosphorolysis-activated Polymerization (PAP) for the detection of the Y chromosome. All fetal gender results were concordant with gender confirmed at birth or after karyotyping in n=194 cases with a sensitivity and specificity of both 100% (CI95% [98.5%-100%]). In n=15 cases results were inconclusive.
To confirm the presence of fetal DNA in plasma in cases with a negative result, we performed maternal discrimination tests. In this study 3 different methods for maternal discrimination testing are compared.
This study shows that fetal gender could be determined from ffDNA in maternal plasma, using a combination of Real-Time PCR and PAP. Although we can detect fetal sex reliably using only these two methods, we do perform a maternal discrimination test for diagnostics in cases with negative results.
This validation study has been completed. As of March 1 2011, fetal sexing is implemented for diagnostics in our department and can be requested.

P05.28
COLD-PCR, a new tool for noninvasive prenatal diagnosis of genetic diseases.

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Following the discovery of fetal DNA in maternal plasma different strategies have been reported for noninvasive prenatal diagnosis of genetic diseases. Despite the advances in improving the analytical sensitivity of methods it is still very challenging to distinguish between fetal and maternal sequences and noninvasive prenatal diagnosis of genetic diseases has not yet attained a clinical routine application. Fetal DNA in maternal plasma is in the presence of a large majority of wild-type sequences of maternal origin which hamper the identification of fetal paternally inherited mutated alleles. To make widely accessible noninvasive prenatal diagnosis we developed conditions for the enrichment of fetal alleles based on co-amplification at lower denaturation temperature-PCR (COLD-PCR).
We designed assays for the identification of two beta-thalassemia mutations, based on full COLD-PCR. In this protocol, heteroduplexes generated between mutant and wild-type sequences were selectively denatured at a critical temperature and amplified allowing the enrichment of fetal mutated alleles which could then be simply detected by direct sequencing. Full COLD-PCR enabled correct identification of fetal paternally inherited alleles in 35 couples.
We provide the first evidence that the application of COLD-PCR enables straightforward and reliable identification of inherited mutated alleles without the need of sophisticated procedures or advanced and costly equipment. Full COLD-PCR can be successfully applied to enrich DNA variants irrespective of the kind and position of the mutation thus providing a powerful tool for noninvasive prenatal diagnosis of fetal alleles in a variety of genetic diseases or in general for the identification of muted minority alleles.

P05.29
Microchip for determination of the syndromes tied to increased Nuchal Translucency

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Increased foetal nuchal translucency (NT) between 11 and 14 weeks' gestation is a common phenotypic expression of chromosomal abnormalities. In the absence of aneuploidy it is associated with adverse perinatal outcome due to foetal malformations and genetic syndromes, including some monogenic disorders. Arrayed primer extension

(APEX) microarray assay was developed to analyse conditions which are related to increased NT in case aneuploidy is excluded. The test includes 3 monogenic disorders (21-hydroxylase deficiency, Smith-Lemli-Opitz syndrome, spinal muscular atrophy), mutations in 5 genes associated with Noonan syndrome and polymorphisms in 22q11.2 region for detection of del22q11.2 syndrome.
Objective: to evaluate the informativeness of the prenatal diagnostic test in case of increased NT in the foetuses with normal karyotype.
Methods: 267 variations in 16 different genes were tested with APEX array in 220 DNA samples isolated from the foetal chorionic cells or amniocytes.
Results: Genetic changes were detected in 24 DNA. In 9 foetuses mutations in gene related to 21-hydroxylase deficiency was detected. Mutations associated with Noonan syndrome were present in 10 foetuses and with Smith-Lemli-Opitz syndrome in 1 foetus. In one foetus homozygosity of the signals in locus 22q11.2 was observed and deletion in this region was suspected. Detailed genotypes and their phenotypic consequences are discussed.
Conclusion: according to our preliminary data the informativeness of our diagnostic test is 9,1% in cohort of the foetuses with NT> 3mm and normal karyotype. This test provides valuable information for the antenatal genetic management of foetuses with increased NT.

P05.30
Ultrasound and molecular prenatal diagnosis of Ophthalmo-Acromelic Syndrome: bowing of the tibia might be an early recognizable feature

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Ophthalmo-Acromelic Syndrome (OAS, MIM 206920) is an autosomal recessive condition mainly characterized by uni- or bilateral microphthalmia or anophthalmia and limb anomalies, especially synostosis of fourth and fifth metacarpals and foot oligodactyly. Recently, loss of function mutations in the SMOC1 gene have been shown to be responsible for the disorder.
We report on a consanguineous couple requesting a prenatal diagnosis of OAS; they have a son (6y) with bilateral anophthalmia, ulnar and fibular reduction defects, bowed tibiae, abnormal Vth fingers, fused IVth and Vth metacarpals, absence of Vth toes and metatarsals, and syndactyly between IInd and IIIrd toes in whom we had diagnosed OAS. Molecular genetic analysis of the SMOC1 gene confirmed the diagnosis identifying the homozygous mutation c.395dupA (p. Tyr132fsX1) of which both parents are carriers.
At 12 weeks and 1 day of amenorrhea (ultrasound corresponding to 11^{+4}) the mother underwent CVS for prenatal diagnosis of OAS and karyotyping. At early morphologic ultrasonography, the lower limbs were bowed and shortened in length, raising the suspicion of distal lower limb defects. Fetal karyotype resulted normal (46, XY), but mutation analysis of fetal DNA revealed the presence of the SMOC1 mutation in homozygosity. Identification of SMOC1 as causative gene will help defining the clinical picture of OAS. This case suggests that a severe lower limb reduction defect with bowing of the tibia can be an early recognizable feature of OAS.

P05.31
Confirmation of genetic outcomes in preimplantation genetic haplotyping (PGH).

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At our PGD centre, 3-day embryos at risk of familial single gene disorders are diagnosed by preimplantation genetic haplotyping (PGH) following whole genome amplification and testing with a panel of STR markers linked to the disease gene. Meiotic microsatellite mutation or PCR "slippage" may compromise diagnosis using such linkage approaches. Confirmation of diagnosis (COD) was therefore carried out on a cohort of embryos following PGH.
Over a one-year period, 49 CODs were carried out using material collected from embryos 1 to 4 days post-biopsy; 9 (18%) were discordant with biopsy diagnosis. One discordance was due to biopsy of a binucleate cell. Of the remaining 8, two were DMD cases with mosaicism for the X chromosome; three CF cases were diagnosed as affected at biopsy, but had a third (low-risk) haplotype at COD; two SMA cases gave aneuploid results with affected haplotypes at biopsy, whilst COD indicated euploid carrier status; one HD case appeared to have monosomy 4 at biopsy, but a normal status at COD. Therefore, these discordances were all likely to be due to mosaicism in the cleavage stage embryos. In only one case (DMD) did COD detect a mutant haplotype not found at biopsy; in this case, the sex chromosome complement was abnormal, and this embryo would therefore not have been considered for transfer. No novel alleles were found in any of these embryos.
These data indicate that neither meiotic mutation nor PCR slippage is likely to compromise PGH diagnosis.

P05.32
New strategy of routine 250K SNP array analysis instead of karyotyping for prenatally diagnosing fetuses with structural ultrasound anomalies

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In October 2010, we implemented a new strategy for routine prenatal diagnosing fetuses with structural ultrasound fetal anomalies (including increased nuchal translucency). We either apply QF-PCR and subsequent 250K SNP array (QF/array), after pre-test counseling and obtaining blood and informed consent from both parents, or QF-PCR and subsequent routine karyotyping (QF/karyo), when parents were not counseled or did not opt for array analysis. Abnormal QF-PCR results were always followed up by karyotyping.
We received 95 fetal samples. In 63/95 (66%), QF/array analysis was requested: 18/63 (29%) were abnormal with QF-PCR, the remaining samples were array analysed. Although this revealed no clinically relevant abnormalities, we detected one paternally inherited gain in 16p13.11 (susceptibility locus), six inherited CNVs and one large contiguous stretch of homozygosity. No incidental, possibly clinically relevant, findings were detected in parental samples. Array analysis was mostly carried out on DNA from uncultured samples and results were often available within 10 days. In 32/95 (34%) fetuses, QF/karyo analysis was performed: 5/32 were abnormal with QF-PCR and 2/32 revealed an abnormal karyotype, not detectable with QF-PCR (but detectable with array analysis).
In conclusion, the QF/array analysis strategy did not reveal additional clinically relevant abnormalities, indicating that, to increase the detection rate, fetuses with more specific fetal ultrasound abnormalities might be selected. Alternatively, as results were mostly available within 10 days and SNP array analysis involves a complete genome analysis with no additional tests needed, one can also opt for implementing the QF/array strategy for all fetuses with structural ultrasound anomalies.

P05.33
Evaluation of specific prenatal ultrasound abnormalities as chromosomal risk factors

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Various fetal abnormalities detected by prenatal ultrasound are well established markers for invasive prenatal diagnosis due to their association with fetal chromosomal aberrations. Aim of this study

was to investigate the correlation of pathologic karyotypes with the number of prenatal ultrasound abnormalities (PUA) and/or certain affected organ systems. 469 pregnancies with suspect ultrasound findings which subsequently underwent invasive prenatal diagnosis were karyotyped. We assigned PUAs to 8 organ specific categories and the impact of quantitative and qualitative subgroup involvement was analyzed.
A pathologic karyotype was found in 18.6% of these pregnancies. An almost linear increase of chromosomal abnormalities with the number of PUAs was found with up to 56% chromosomal abnormality rate when 5 or more PUAs were present. Multiple subgroup involvement showed even higher rates (62%). Single detected PUAs within the subgroups were qualitatively analyzed and the highest incidence of pathologic karyotypes was found amongst single detected hygromas or hydrops (39%). Single detected PUAs in all other subgroups were only rarely associated with a chromosomal abnormality (2.4%). The most frequent, single reported PUAs were choroid plexus cysts and ventriculomegaly with less than 1% chromosomal abnormality rate.
Due to the high impact of the number of detected PUAs and the number of affected organ systems on chromosomal abnormality rates any detection of PUAs should lead to an extensive screening for further fetal anomalies. If certain abnormalities such as choroid plexus cysts and ventriculomegaly are found solitarily, the risk for chromosomal abnormalities remains low.

P05.34
Prenatal detection of numerical chromosomal aberrations in Institute for Children and Youth Health Care Institute of Vojvodina in last 12 years

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In this paper we present incidence of prenatally detected aneuploidies during last twelve years (1999-2010) in Medical Genetic Centre in Novi Sad, Vojvodina, northern part of Serbia. We used conventional cytogenetic analysis (G banding technique) for detection of chromosomal abnormalities. Samples were obtained from women in risk for chromosomal abnormalities, who underwent amniocentesis between 16th and 19th week of gestation or cordocentesis between 20th and 22nd week of gestation.
We performed retrospective study for aneuploidies on 22847 amniotic fluid and fetal blood samples. We detected 376 cases of numerical aberrations at all. We detected autosomal trisomy in 243 cases; gonosomal aneuploidies in 110 cases; polyploidies in 19 cases and 4 monosomies.
Amniocentesis and cordocentesis was recommended for pregnant women over age 35, women who had an abnormal fetal ultrasound result, women who had an abnormal ,,double screen“ or “triple screen” biochemical screening test during first or second trimester of pregnancy, or women who had (or whose husbands had) a family history of certain diseases, chromosomal abberations or birth defects. Couples had opportunity to choose termination of pregnancy or continue the pregnancy after invasive prenatal diagnosis.

P05.35
Prenatal diagnosis of 48,XXX,+18 double trisomy

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Double trisomy (DT) is a rare event. Commonly, pregnancy ends in miscarriage. Pregnancies that continue beyond the first trimester are rare and have DT involving one sex chromosome in combination with potentially viable autosomal trisomies such as 21, 18 and 13.
We present a case of DT, 48,XXX,+18, diagnosed because of sonographic findings in the second trimester.
FISH analysis was performed in the first time showing 3 chromosomes 18 and 3 chromosomes X. karyotyping confirmed the DT.
Parents elected to terminate the pregnancy and fetal autopsy confirmed the sonographic findings.
DT is rare event. Only few pregnancies continue. Ultrasounds controls are important to discover fetal malformations and perform karyotype.

P05.36
Dual testing with QF-PCR and karyotype analysis as standard approach of prenatal diagnosis of chromosomal abnormalities. Evaluation of 13,500 cases in Greece.

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Last years, the need for rapid prenatal diagnosis of the most common fetal aneuploidies and improvement of pregnancy management provoked the development of a reliable and cost-effective method known as Quantitative Fluorescent PCR (QF-PCR), to be used in a dual test with conventional karyotype analysis. In this study, we report our experience of dual prenatal testing with QF-PCR and karyotype analysis in 13,500 cases. The main aim of the study is to examine the detection rate of clinically significant chromosomal abnormalities by QF-PCR compared to karyotype analysis and assess the necessity of dual prenatal testing in all cases, regardless indications of referral. The rates of concordant results between the two methods were evaluated. Abnormal karyotype was found in 325 out of 13,500 cases (2.4%). From these, QF-PCR did not detect the abnormality in 70 cases (21.5%), while 34 (10.5%) had a high or unknown risk of adverse outcome. By selectively applying dual testing only at cases with ultrasound findings and/or genetic history, 20 out of these 34 would have been correctly diagnosed. Selective dual testing would lead to prenatal diagnosis of normal and high risk abnormal karyotypes in 13,483 out of the 13,500 pregnancies (99.87%). Our study strengthens the shift towards selective dual testing in the presence of ultrasound findings and/or genetic history, and QF-PCR as stand alone test for the rest of the cases. However, ethical issues still exist and further evaluation of more studies would be needed for final conclusions.

P05.37
Prenatal diagnosis of chromosome abnormalities by quantitive fluorescent PCR in North-West Russia

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Prenatal diagnosis for the most common chromosome abnormalities, such as Down’s, Edwards’, Patau’s syndromes and also numerical sex chromosomes abnormalities has been carried out by quantitative fluorescent PCR (QF-PCR). QF-PCR relieves anxiety of most parents within 24 hour from sampling and accelerates therapeutic interventions in the case of an abnormal result. The study summarizes our experience of prenatal diagnosis for chromosome abnormalities by QF-PCR. 17 STR markers (D21S11, D21S1437 D21S1411, D21S226, D13S628, D13S634, D13S742, D18S380, D18S386, D18S391, D18S535, AMXY, DXS981, DXS6854, X22, P39, XHPRT) were used.The heterozygosity value for each chromosome-specific marker was determined. The use of 3 to 4 markers for each chromosome increases heterozygosity and informative value of QF-PCR assay up to 99-100%. From all 763 samples of fetus tissue 32 trisomic cases, including 22 cases of trisomy 21, 8 case of trisomy 18, 2 case of 47 XXY, 2 case of 45X and 1case of 69 XXY were identified. Submicroscopic polymorphic duplications of microsatellites were observed in 7 cases as clear trisomic triallelic or diallelic patterns for one chromosome-specific STRs. Results of molecular study were verified by conventional cytogenetic analysis. QF-PCR can be suggested as a fast, simple and reliable method of prenatal diagnosis of common chromosome diseases.

P05.38
Detection of STR duplications in prenatal diagnosis of chromosome abnormalities by quantitative fluorescent PCR

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The quantitative fluorescent PCR (QF-PCR) assay, introduced during the last few years, allows prenatal diagnoses of common chromosome aneuploidies within a few hours after sampling. In the past 2 years, we have tested 560 prenatal samples for trisomies 13, 18 and 21 using a quantitative fluorescence-PCR (QF-PCR) approach. Submicroscopic polymorphic duplications of microsatellites were observed in 7 cases

as clear trisomic triallelic or diallelic patterns for one chromosome-specific STRs. Duplications were observed in one sample for one STR on chromosome 21(D21S1437), in five cases for one of the markers on chromosome 13 (3 cases for D13S634 and 2 cases for D13S742) as well as for D18S391 marker of chromosome 18. The maternal origin of the duplication could be demonstrated in 6 cases by QF-PCR analysis of the same marker in both parents; in all samples the polymorphism was found as inherited from the mother. In one sample the duplication was found to be de novo.
The finding of submicroscopic duplications in microsatellites should also be a warning about the risk in reporting an autosomal trisomy detected with a single informative STR. While the same result could also be due to partial trisomies (i.e. unbalanced translocation), the analysis of both parents with the same marker allowed us to distinguish the rare inherited polymorphism in 6 out of 7 cases.

P05.39
" A deletion in 18 chromosome with QF-PCR"

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The diagnosis of β-thalassemia relies on measuring red blood cell indices that reveal microcytic hypochromic anemia, nucleated red blood cells on peripheral blood smear, hemoglobin analysis that reveals decreased amounts of HbA and increased amounts of HbF after age 12 months, and the clinical severity of anemia. Molecular genetic testing of the gene encoding the hemoglobin subunit beta (*HBB*) is available in our laboratory and may be useful for predicting the clinical phenotype in some cases as well as presymptomatic diagnosis of at-risk family members and prenatal diagnosis.
Prenatal diagnosis is possible when both parents are carriers.
Our protocol consists in: Fetal DNA extraction from chorionic villi sampling at 11-12 weeks' gestation; detection of parental mutations with Reverse Dot Blot Analysis (RDB) and/or direct sequencing; the potential presence of maternal cell contamination (MCC) in fetal samples is detected by Quantitative Fluorescent-Polimerase Chain Reaction (QF-PCR).
Here we report a case of prenatal diagnosis in which both parents were carriers of the nonsense mutation Codon39. The fetus resulted heterozygote Codon 39. QF-PCR showed no MCC but revealed a missing paternal correlation for the microsatellite D18S386.
Fetal and parental samples underwent array Comparative Genomic Hybridization (aCGH) to detect a possible microdeletion in that locus. This analysis confirmed a 147 kb deletion at 18q22.3 in the propositus inherited from the father, and so we can exclude any patological meaning.
The QF-PCR test could allow not only identification of MCC in the fetal material but also detection of chromosome rearrangements (13,18, 21 and sexual chromosome).

P05.40
Unexpected findings using QF-PCR as a rapid test for aneuploidy detection in prenatal diagnosis

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Rapid aneuploidy testing in prenatal diagnosis using MLPA or QF-PCR with markers for the chromosomes 13, 18, 21 and XY is now being widely used. Arguments for using these tests are the short reporting time, high throughput and low costs. Additionally, unexpected findings as seen in full karyotyping and leading to difficult counselling issues, which cause anxiety for the parents, can be avoided. The tests may be used in combination with karyotyping or as a standalone test for specific indications such as advanced maternal age or increased risk for Down syndrome.
In 2010 a total of 397 amniotic fluid samples were analysed in our laboratory with QF-PCR, using Aneufast kit S1/S2 and M13, M18, M21 and MXY (Genomed Ltd, Kent, UK), followed by karyotyping. QF-PCR yielded unexpected findings in two cases: in one case the two most distal markers on the long arm of chromosome 18 were trisomic. Additional karyotyping showed an unbalanced translocation (47,XX,+der(9) t(9;18)). The second case showed a mosaic 46,XX/46,XY, but no peak was seen for the SRY-marker. Karyotyping showed a 45,X cell line and a cell line with an additional small derivative Y-chromosome.
We report two cases of unexpected findings with QF-PCR in prenatal diagnosis. We conclude that, when offering QF-PCR as a standalone test, unexpected findings are not completely excluded and should be discussed with the patient. In a minority of cases, full karyotyping will still be necessary to enable appropriate prenatal counselling on the significance of the QF-PCR results.

P05.41
The development of a rapid assay for prenatal detection of common aneuploidies and microdeletion syndromes

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Fluorescence *in situ* hybridization (FISH) testing on uncultured cells has been widely used as a rapid test to identify the common aneuploidies in prenatal samples. We have developed a novel assay utilizing Luminex xMAP® technology for the evaluation of 15 microdeletions in addition to the common aneuploidies for use in low-risk pregnancies. We selected microdeletion syndromes that are not easily detectable by karyotype analysis and that are not usually associated with an abnormal ultrasound. Our validation involved 430 samples including both direct and cultured CVS and amniotic fluid samples, as well as samples diluted with normal control DNA to simulate maternal cell contamination (MCC) and mosaicism. Four investigators reviewed the results and correctly identified all microdeletions and aneuploidies. The only exceptions were a 69,XXX, which cannot be distinguished from a normal female by this assay, and a normal male with ~20% MCC that was incorrectly interpreted as 69,XXY. In experiments simulating MCC, the admixture of normal female cells became apparent at a level of 20%-30% MCC, although abnormalities were still evident up to 45-50% MCC. Simulated mosaicism for trisomy 21 showed detection of the abnormal cell line down to a level of 30-35%. Below this level, the abnormal cell line was not consistently identified. We have developed an alternative to FISH aneuploidy screening and microarray analysis in low-risk pregnancies undergoing invasive testing. This assay is likely to be preferred by women who seek testing beyond routine karyotyping and aneuploidy FISH but are reluctant to consider microarray analysis.

P05.42
Unbalanced reciprocal translocations identified at prenatal diagnosis

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Balanced reciprocal translocations, which are typically an exchange of two terminal segments from different chromosomes, occur in approximately one in 500 live births, and are usually associated with a normal phenotype. Reciprocal translocations may be associated with infertility in some carriers, while all carriers have reproductive risks due to abnormal segregation of the translocation chromosomes at meiosis, resulting in sperm or eggs with chromosome imbalance.
We present three types of unbalanced reciprocal translocations detected prenatally using G-banding, fluorescent *in situ* hybridization and spectral karyotyping. The first two cases represent partial monosomy 4q and partial trisomy 4q due to a balanced translocation 4q;21p in the mother. Isolated 4q duplications result in variable clinical features, often including growth and developmental delay and dysmorphic appearance. Patients with 4q terminal deletions share enough similarities to represent an identifiable phenotype. Findings in these patients include craniofacial anomalies, congenital heart and genitourinary defects, moderate to severe mental retardation, poor postnatal growth, and hypotonia. In third case we report an unbalanced segregation of a maternal translocation t(3;8)(p26;p22) causing partial trisomy 3p/partial monosomy 8p. It was the fifth pregnancy of the mother with four miscarriages and fetal ultrasound showed a hypoplastic left heart. There are described in the literature recurrent deletions of a region of 8p23.1 which are associated with congenital heart defects.
Couples carrying reciprocal translocations may request preimplantation genetic diagnosis in order to select embryos with no genetic imbalance and hence increase their chances of a successful pregnancy.

P05.43
Identification of a novel common microdeletion within the *RHD* gene in 56 French patients: implication for prenatal diagnosis

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In the field of blood transfusion, the accurate typing of *RHD* alleles is of major clinical relevance to prevent from alloimmunizing *RHD*- recipients with blood from *RHD*+ donors. In ~1-2% of cases, discrepancy in the phenotypical typing of rare *D* alleles may occur, and a molecular analysis of the *RHD* genotype is therefore required. From January 2003 to July 2010 more than 2,000 blood samples presenting ambiguity for D phenotype by routine serologic analyses were collected in laboratories from the French Blood Centre (Etablissement Français du Sang) in several French regions. Since the amplification of *RHD* exon 10 could not be achieved by a conventional PCR method in 56 blood samples, we designed a protocol to investigate the region that was suspected to be deleted. We report the identification of a 5.4-kb microdeletion in all samples, including the whole sequence of exon 10, which contains the stop codon. Although the transcript that is generated is suspected to be aberrant and thus degraded, a substantial amount of protein appears to be biosynthesized and some antigens may be partly recognized by anti-D specific antibodies, then potentially conferring a weak D phenotype. This finding raises the question of noninvasive method for fetal *RHD* typing by amplification of exon 10, which is supposed to be negative while an aberrant, antigenic protein is nonetheless produced. Subsequent molecular analyses are currently in progress to investigate whether this haplotype derives from a common ancestor, or if the breakpoint is a mutation hotspot.

P05.44
The fetal RHD genotyping from cell-free fetal DNA circulating in maternal blood - The first non-invasive prenatal test in Romania

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Background: Detection of cell-free fetal DNA in maternal circulation, the new non-invasive prenatal diagnosis (NIPD) approach, raised the challenge of finding safer methods for prenatal diagnosis.
We report the first study of non-invasive fetal RHD genotyping performed in Romania.
Objective: The prenatal diagnosis procedures currently employed in Romania are based exclusively on invasive sampling procedures of fetal cells. Our aim was to develop a protocol for non-invasive fetal RHD genotyping in pregnancies at risk: the pregnant woman is RhD negative and her partner is RhD positive.
Methods: We developed a three-step method for fetal RHD genotyping and we tested 56 pregnant RhD negative women with RhD positive partners. The cell-free total DNA extraction was performed from 1ml maternal plasma using the QIAamp® DSP Virus kit with some adjustments to the standard QIAGEN protocol. We performed the fetal RHD genotyping using a commercial 2x multiplex PCR master mix and specific primers for two sequences in the exon 5 and respectively exon 7 of the RHD gene. We also included the beta-globin sequence detection as internal control. The PCR products were automated analyzed by high resolution capillary electrophoresis.
Results: We detected 39 fetuses with RHD positive genotype and 17 fetuses with RHD negative genotype. All 56 cases were analyzed in triplicate and the results were confirmed by the invasive method.
Conclusion: Our results confirm that this non-invasive approach is feasible and accurate and improve the management of mother-fetus RhD incompatibility and isoimmunization by eliminating the invasive procedure for fetal RHD genotyping.

P05.45
Presence of a partial RH1 by fetal genotyping RHD in maternal plasma: Clinical and biological expression at birth. About a case

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Introduction: mrs. B. Marie, caucasian, 5th pregnancy, presents a poly-alloimmunization already known during a previous pregnancy: a non evolutive anti-RH1 + an anti-RH2
+ an anti-JK1.
fetal RHD genotyping in maternal plasma performed at 23 weeks and 33 weeks show an amplification of exon 10, but no amplification of exons 4 and 5. Maternal buffy coat showed no amplification. It may be a partial D inherited his African father (RHD gene no analyzed) .
The birth takes place at 37 weeks: at birth, the child has no anemia, moderate hyperbilirubinemia quickly contained by phototherapy, no transfusion was necessary.
Results and hypothesis: the direct antiglobulin test is positive at birth. Elution test finds the presence of anti-RH2, anti-JK1 and anti-RH1 while the newborn is Rh: -1.2 and JK: 1. The sequencing of the RHD gene shows an aspect of partial D associated with a deletion of some exons: how is the expression profile? It may be a nonfunctional gene and anti-RH1 found in the eluate is present in the free state, in the close environment of the erythrocyte (Matuhasi-Ogata phenomenon). Conversely, in case of a gene with partial antigen expression, the result of RH1 phenotype at birth may be due to saturation of antigenic sites by corresponding antibodies.
Conclusion: a new sample a few month after birth would confirm its RH1 phenotype.

P05.46
Fetal RHD genotyping in maternal plasma: Detection of variantsforms

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Background and aims: the prenatal determination of fetal RH1 represents an important tool to guide the management of pregnancy in severe maternal alloimmunization context or to indicate an immunoprophylaxis during pregnancy of non anti-RH1 allo-immunized women.
Normal and partial RH:1 phenotype can induce or re-activate an anti-RH1 allo-immunization: It's therefore important to detect wild and variant forms of RHD gene, this capacity is determined by the choice of primers and probes.
Methodology: exons 4, 5 and 10 of RHD gene are analysed on free fetal DNA containing maternal plasma after 10 weeks of pregnancy by quantitative real-time PCR using TaqMan technology.
Variant forms are suspected if non homogeneous amplifications of the three exons are observed, like the pseudogene Ψ by Africans. Buffy-coat of one or the two parents are tested following the Ct values obtained by fetal genotyping. Variant RHD gene can be sequenced to study its functionality.
Results: all variant forms of RHD gene found in our laboratory show amplification of at least one but not the three tested exons.
Conclusions and prospects: buffy-coat amplification profile of a few blood donnors in Rhône-Alpes with sequenced variant forms of RHD gene are currently studied to verify the specificity of the method.

P05.47
Seckel syndrome diagnosed prenatally and confirmed postnatal

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Seckel syndrome is a rare form of primordial dwarfism, characterized by severe intrauterine and postnatal dwarfism, severe microcephaly with variable mental retardation, facial anomalies and skeletal abnormalities.
We report a case of Seckel syndrome diagnosed prenatally by ultrasound and confirmed postnatal. Ultrasound examination at 20 weeks of gestation revealed the following parameters on the lower growth curve corresponding to fetus age: biparietal diameter, head

circumference, abdomen circumference, occipitofrontal diameter, femur length, estimated fetal weight. The examination at 26 weeks of gestation showed severe intrauterine growth retardation. The suspicion of Seckel syndrome was raised, corroborating the growth parameter data with the typical facial signs: beak-like protrusion of nose and micrognathia.
A male infant was delivered by caesarian section at 40 weeks of gestation, with weight of 1520 g; length of 39 cm; cranial circumference of 26 cm. On examination we noticed characteristic narrow face, receding forehead, large eyes, prominent beaked nose, micrognathia, low-set ears. Other manifestations were: closed anterior and posterior fontanelles, clinodactyly of fifth finger, supernumerary nipple on the left side and criptorchidism. Thoracic X-ray showed only 11 ribs, while cranial X-ray revealed sinostosys of sagittal and metopic sutures. MRI revealed smaller cerebral hemisphere volume, enlarged lateral ventricles, cerebellar hypoplasia**.** The diagnosis of Seckel syndrome was set based on postnatal typical clinical and imagistic findings. The patient will be followed up to asses the development of mental status for a correct management.
Seckel syndrome should be considered as possible diagnosis when prenatal ultrasound reveals severe microcephaly and intrauterine growth restriction.

P05.48
Fetal presentation of Silver Russell syndrome

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Silver-Russell syndrome (SRS) is characterized by intrauterine (IUGR), postnatal growth restriction, relative macrocephaly and typical dysmorphic features. Two mechanisms account for 70% of SRS cases: hypomethylation of 11p15.5 imprinting center region 1 (ICR1) and maternal uniparental disomy for chromosome 7 (matUPD7). Prenatal diagnosis is possible only for matUPD7.
We report the antenatal and postnatal data of three unrelated cases of SRS. Pregnancy was marked by asymmetric IUGR sparing the head at 22 weeks of gestation (WG) with normal prenatal karyotype. Cesarean section delivery was induced due to cessation of fetal growth at 32 WG (case 1) and at 30 WG (case 2) while case 3 was stillbirth at 27 WG. Postnatal echocardiography detected blocked supracardiac total anomalous pulmonary venous return (TAPVR) in case 1 and hypoplastic aortic arch and cardiac TAPVR in case 2. Both died shortly after birth. Autopsy (refused in case 1) findings were: multivisceral hypoplasia (case 2), genital and extremity anomalies (case 3). Hypomethylation of ICR1 11p15.5 was identified in the three cases and confirmed the clinically suspected diagnosis of SRS.
SRS should be considered in cases with second trimester asymmetric IUGR sparing the head. Notably, the association of SRS and lethal cardiopathies is rarely reported. Either they represent two distinct pathologies or SRS might be underdiagnosed when associated with lethal cardiopathies. Finally, IUGR, cardiopathies and prematurity led to death of cases (1 and 2). Should premature delivery in SRS be avoided to improve prognosis? A reliable prenatal diagnosis for SRS would prove to be useful.

P05.49
Sirenomelia and caudal malformations in two families.

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Sirenomelia is a rare fatal condition characterized by abnormal fusion of the lower extremities. Sirenomelia is usually sporadic, and monozygotic twins are usually discordant (Stocker, 1987). Familial recurrence of sirenomelia is exceptional, with only one family reported, with two sirenomelic sibs, another with renal agenesis and the last with imperforate anus (Rudd and Klimek, 1990). In monozygotic twinning, concordant twins have been described (Roberge, 1963), and semi-concordants (Martinelli et al., 1982; Akbiyik et al., 2000). Sirenomelia with renal and anal anomalies in other siblings has been described once (Selig et al., 1993). We report two additional families. In the first family, the mother was operated of a short form of imperforate anus. Her first pregnancy was terminated because of presence of bilateral renal agenesis with anamnios. Her second pregnancy was interrupted because of sirenomelia. The second family was referred because of the occurrence of caudal malformation in their two children. Parents have normal X-rays of the spine. The first pregnancy gave birth to a girl with imperforate anus. X rays showed absent S3-S5 and coccyx. CT-scan of the pelvis showed abnormal pelvic floor with poor musculature, mostly fibrous fatty content without tendinous attachments. The bladder was very anterior and almost bifid inferiorly. A bifid uterus was present. The second pregnancy gave birth to a baby girl with sirenomelia. No diabetes were present in the pregnancies. Molecular studies are underway to better elucidate the genetic basis of sirenomelia in human.
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P05.50
Prenatal diagnosis of tetrasomy 5p by CVS and amniocentesis

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We report the prenatal diagnosis of a fetus with tetrasomy 5p resulting from a supernumerary isochromosome for the short arm of chromosome 5 detected in all cells obtained from chorion villous sampling preformed for a positive combined first trimester screening test with an increased nuchal translucency at a maternal age of 42 years. An amniocentesis revealed the same finding in a mosaic with 7% aberrant cells by a standard karyotyping and 20% by FISH. The pregnancy was terminated. The fetus had some dysmorphic features-hypertelorism, a wide nasal bridge, microgantia, small earlobes, a hypoplastic right upper lung lobe and a bicornate uterus. The examination of fibroblasts revealed the mosaic of isochromosome 5p in 13% of mitosis by karyotyping. Isochromosome 5p seems to be present relatively frequently as a confined placental mosiacism, but when an aberrant ultrasound finding is detected there is a higher risk of true mosaicism, as was in our case. CVS findings need to be verified by amniocentesis. A parental age effect has been reported with the formation of izochromosomes, which is also supported by our findings.

P05.51
A case of co-inheritance of alpha and beta thalassaemia with almost normal haematological indices: an alert for carrier screening

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Thalassaemias are a group of monogenic disorders characterized by reduction of one or more globin chain synthesis.
Prevention of haemoglobinopathies, mainly through prenatal diagnosis, is the major strategy against expansion of the disease. Prevention is based upon carrier identification based on red cell indices and HbA2 values. The high incidence of α- and β- thalassemia traits in Greece often results in co-inheritance of both mutant aplotypes. In practically all cases, the haematological phenotype of a double heterozygote for both α- and β-thalassemia is similar to the one of a β-thalassemia carrier except from an increase in MCV values.
We present here a quite interesting rare case of an α- and β-thalassemia carrier whose erythrocyte indices are far from characteristic of the

underlying beta globin gene mutation. An adult pregnant female was screened for thalassaemia and presented the following values: Hb:11,7g/dL; HCT:34,6%; RBC:4,29x10^6/ml; MCV:**80,7**fL; MCH:**27,3**pg and HbA_2:**4,1**%. Subsequent molecular genetic analysis, due to the increased HbA_2, revealed that the individual was a double heterozygote for both α- and β-thalassemia (beta globin gene: IVS-1-6 T->C substitution and alpha globin gene locus: -α3.7 deletion). Occasionally, carriers of IVS-I-6 and IVS-I-110 with only mild reduction in MCV and MCH values have been observed. In such cases, co-inheritance of a-thalassaemia, as in the herein reported case, might further improve erythrocyte indices, near to normal, a fact which increases the risk for misdiagnosis. In conclusion, we propose that in similar cases identification of the underlying mutations is mandatory in order to avoid misdiagnosis.

P05.52
National Thalassemia Prevention Programs in the Middle East Muslim countries with emphasis on Iranian experience

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Thalassemia is associated with people in Iran, Pakistan, Arab countries around Persian Gulf (Oman, Bahrain, UAE and Saudi Arabia), Egypt, Syria and Turkey. Thalassemia has high burden on health resources and has raised the need to adopt community-based prevention program. This may include public awareness, population screening, genetic counseling, and PND to reduce the incidence of thalassemia. Establishment of a comprehensive screening and prevention program faces many problems when prenatal diagnosis and termination of pregnancy is not permitted or financial resources are limited. In Iran, National Program for the Prevention of Thalassemia was implemented in 1997. This led to a sharp drop on the birth of affected children with thalassemia and sickle cell disease during the past 13 years by providing help to those couples who were both carriers. After this experience, Jordan, Saudi Arabia and Bahrain implemented similar programs for thalassemia and SCD in 2001, 2002 and 2004 respectively. UAE and Oman are taking similar measures. Program in SA and Bahrain lacks prenatal diagnosis component, therefore one does not expect sharp drop in the birth of affected children unless carrier couples are guided to go to other countries for therapeutic abortions. In SA, in 1990, a Fatwa “allows termination of pregnancy in the first 120 days after conception if the fetus is shown beyond doubt to be affected with severe malformation that is not amenable to treatment.” Bahraini government also has stated that they have noticed sharp drop on the birth of SCD after the implementation of the program.

P05.53
Two prenatal cases of 22q11 duplication detected by SNP array

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The 22q11 region is predisposed to rearrangements. The 22q11.2 microdeletion syndrome is the most common. We report two prenatal cases of 22q11 duplication detected by Illumina SNP array using HumanCytoSNP-12v2.1. Generated data were analysed by Illumina KaryoStudio 1.3 and GenomeStudio V2010.2.

Case 1: In the foetus with ultrasound detection of agenesis of the corpus callosum 22q11.2 duplication was found. The 22q11.2 duplication phenotype is extremely variable. According to our knowledge it is the first report of a case 22q11.2 duplication associated with such type of the abnormality of central nervous system.

Case 2: In the foetus with abnormal ultrasound findings including high probably rectal atresia and agenesis of one umbilical artery marker chromosome was detected. Using SNP array the marker chromosome was identified as 22q11.1 duplication overlapping the critical region for cat-eye syndrome (CES).

The SNP array together with other molecular cytogenetic techniques is very useful in identifying of the small structural abnormalities of foetal chromosomes. This is important to specify prognosis of an affected foetus.

P05.54
Role of prophylactic therapy in inherited thrombophilia - study on a group of patients in Oncomed medical center

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Thrombophilias are hereditary or acquired conditions that determine a hypercoagulable state predisposing to thromboembolic events. The aim of our study was to prove the efficiency of thromboprophylaxis in pregnant women with hereditary thrombophilia.

Methods: A total of 41 women with inherited thrombophilia were followed in the medical center ONCOMED Timisoara. 14 patients had a MTHFR-PAI mutation, 3 women were homozygous for the C677T MTHFR mutation, 3 were homozygous for the A1298C MTHFR mutation and 2 were heterozygous for this mutation. MTHFR heterozygous mutation combined with the factor V Leiden mutation was observed in 6 patients. The prevalence of protein C deficiency has been found in 5 patients, the protein S deficiency in 4 women and the combined deficit for protein C and S**,** in 2 patients. 2 patients presented a heterozygous prothrombin mutation. All women with heterozygous mutations received aspirin 75 mg/day and folic acid 5 mg/day during pregnancy whereas those with homozygous mutation, low molecular weight heparins (dalteparin) 5000IU/day.

Results: We found no significant differences between the groups related to the incidence of congenital malformations or abortions, intrauterine growth restriction or preterm deliveries. 2 women in dalteparin group gave birth to a child with patent ductus arteriosus. 3 women in the same group had heparin induced thrombocytopenia. There were no cases of pregnancy related thrombosis in either group and all women had a live birth.

Conclusion: Thromboprophylaxis in pregnant women with hereditary thrombophilia has an important role in ensuring a normal pregnancy and a live birth for these women.

P05.55
Fetal Akinesia in Metatropic Dysplasia: Overlap between Chondrodysplasia and Neuropathy?

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Heterozygous mutations in the TRPV4 gene have been associated with a family of skeletal dysplasias (metatropic dysplasia, Pseudomorquio type 2, SMD Kozlowski, and brachyolmia) as well as with dominantly inherited neuropathies (hereditary motor and sensory neuropathy 2C, scapuloperoneal spinal muscular atrophy (SMA), and congenital distal SMA). While there is phenotypic overlap between the various members of each group, the two groups were considered to be separate with the former being strictly a structural skeletal condition and the latter group being confined to the nervous system. We report here on fetal akinesia as the presenting feature of severe metatropic dysplasia, suggesting that certain TRPV4 mutations can cause both a skeletal and a neuropathic phenotype.

Case 1 was diagnosed at week 20 with short limbs, absence of limb movements, elbow pterygia, and finger contractures. Cases 2 and 3 (twins) were observed to have short limbs with arthrogryposis and akinesia at week 20. Both pregnancies were interrupted and fetal radiographs suggested metatropic dysplasia. Case 4 presented with joint contractures and absent limb movements at birth; skeletal survey showed typical features of metatropic dysplasia. The baby died of respiratory complications at age 4 months. Sequencing of the TRPV4 gene confirmed the presence of de novo heterozygous mutations predicting G78W (case 1), T740I (cases 2 and 3), and K276E (case 4). Although some degree of restriction of movements is not uncommon in fetuses with skeletal dysplasia, akinesia as leading sign is unusual and may indicate that certain TRPV4 mutations produce a severe “overlap” phenotype.

P05.56
Congenital diaphragmatic hernia associated with a bifid tongue and cleft palate: a case report.

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Objective: We describe a female infant, in whom a left congenital diaphragmatic hernia, a bifid tongue, cleft palate and other congenital anomalies coexisted.

Case report**:** A 39-years-old female, G5P2, had undergone regular prenatal care. She underwent amniocentesis at 16^{+6} weeks of gestation. The result showed normal female karyotype (46, XX). Fetal growth was appropriate throughout the pregnancy. At 20 weeks, ultrasound scan revealed a bifid tongue and a left congenital diaphragmatic hernia; no other abnormalities were evidenced. A genetic consultation was obtained after these findings. At birth, the baby showed respiratory distress and poor respiratory functions, for that she was immediately intubated by the pediatrician and sent to the intensive care unit for further evaluation. After few days, the baby underwent surgical intervention for the diaphragmatic hernia. Clinical evaluation has demonstrated the presence of pedunculated lingual mass, soft mass arising from the palate and cleft palate. Histological examination of the excised tissues from the revealed masses is ongoing. A blood sample was withdrawn from the baby to perform CGH-array in our laboratory. This analysis is still ongoing.

Conclusion: These conditions, independently, may be isolated or associated with other congenital anomalies; however, to our knowledge, their coexistence in the same patient is not described before.

J05.01
Prenatal diagnosis for hemophilia A using Inverse-shifting PCR in an Iranian family

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Hemophilia A (HA) is an X -linked coagulation disorder with a world-wide incidence of approximately 1 in every 5000 males. Almost half of the patients with severe HA have large inversions that disrupt either intron 22 (Inv22) or intron 1 (Inv1) of the factor VIII (F8) gene. The identification of carriers and the prenatal diagnosis of Inv22 and Inv1 have greatly improved with new strategies. Recently, inverse shifting-PCR (IS-PCR) has been developed as a new diagnostic test. Here we report the reproducibility of the modified method in prenatal diagnosis of Inv22 in an obligate carrier with a male fetus at 13 weeks of gestation. After determination the carrier status of the mother, genomic DNA of chorionic villus sample (CVS) was dissected and extracted according to standard protocols. Genomic DNA was digested by BclI, followed by self-ligation to create BclI circles. PCR was performed on circular genomic DNA. Our results confirmed Inv 22 is responsible for the disease in the family and fetus was normal. Prenatal diagnosis offers an option, namely to restrict abortions to hemophilic fetuses only, and thus retain the chance of bearing normal sons. It seems IS-PCR technique have proven to be a rapid, robust and reliable technique for genotyping of inv22 in HA patients, carrier detection and prenatal diagnosis.

J05.02
Prenatal screening for triploidy using nuchal translucency and maternal serum pregnancy associated plasma protein A in first trimester of gestation.

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Triploidy has been estimated to occur in 1-2% of all clinically recognized conceptions. It has been assessed that only 1/3 of triploid conceptuses survive post 15 weeks gestation.

There are two types of triploidy. In type I, where the additional chromosome set is of paternal origin, the placenta is partially molar and the fetus is relatively well-grown. Type II, where the extra chromosome set is of maternal origin, is characterized by a small normal looking placenta and severe asymmetrical fetal growth restriction.

We present a case of triploidy discovered by amniocentesis which was carried out at 16 weeks gestation in a 25-year-old woman, following an abnormal maternal serum screening test at the first trimester of gestation and fetal nuchal translucency (NT) thickness was significantly increased (1.97 MoM).The maternal serum markers used were free human chorionic gonadotrophin (freehCG), and pregnancy associated plasma protein A. The fetus was identified as screen-positive for Edward's syndrome (trisomy 18), with very low level PAPP A (MoM = 0,15), and normal hCG levels. Ultrasound findings showed at 16 week of gestation early intrauterine growth retardation and severe oligohydramnios. To identify any possible chromosomal abnormality, cytogenetic investigation was carried out on the amniotic fluid sample. The fetus's karyotype showed triploidy with 69, XXY chromosome complement in all the metaphase spreads obtained from two different cultures, using GTG banding technique.

Triploidy could be identified in the first trimester using NT, maternal serum free beta-hCG and PAPP-A.

J05.03
Prevalence of RFC - 1 A80G polymorphism in romanian mothers having children with Down Syndrome

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The *RFC1* (reduced folate carrier protein) gene is located on chromosome 21 and likely overexpressed in trisomy 21 individuals. The polymorphisms of the genes involved in folate metabolism may be associated with higher risk of Down syndrome (DS) pregnancy.

Our study included 26 women who gave birth to DS babies and 46 control mothers of healthy children. All women in our study reside in the same geographic area and have a similar social background. The DS mothers were younger than 40 years old and 7 of them had a history of spontaneous miscarriages (26.92%). Genomic DNA was isolated from whole peripheral blood collected on EDTA, using peqGOLD blood DNA mini kit (ATP Biotech). The *RFC1* A80G mutations were investigated by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis.The results show that there was no significant difference in heterozygous genotype frequencies between the two groups. Moreover, while AA homozygous genotype frequency was higher among control mothers than among DS mothers (10.87% versus 3.85%), the overall combination of GA heterozygous and AA homozygous *RFC1* variant genotypes did not show significant difference between the case and control groups (OR 0.59 [0.21-1.70] P 0.33). This is the first study that has analyzed the *RFC1* A80G polymorphism as a maternal risk factor for DS, in a cohort of Romanian mothers of DS children, in comparison with control mothers.

J05.04
MTHFR gene polymorphism as a maternal risk factor of Down Syndrome in romanian population

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Methylenetetrahydrofolate reductase (*MTHFR*) is an important enzyme involved in folate metabolism; it catalyzes the conversion of 5,10-methylenetetrahydrofolate (5,10-MTHF) in 5-methyltetrahydrofolate (5-MeTHF), the latter representing the active form of folate that is involved in remethylation of homocysteine to methyonine.

MTHFR polymorphism involves an adenosyne to cytosine substitution at base pair 1298 (A1298C), causing a glutamate to alanine substitution in the MTHFR protein. The polymorphism is located in exon 7, within the presumptive regulatory domain. The present study includes 72 women (ages 20-42 years old): 26 of them, that gave birth to DS children, citogenetically confirmed as regular trisomy 21, including 7 women with a history of spontaneous miscarriages, and 46 control mothers that gave birth only to healthy children, without any history of miscarriages or abnormal pregnancies. All genotype analyses were performed using PCR-RFLP.CC homozygous genotype frequency at position 1298 was higher in DS mothers than in controls (19.2 % versus 6.5% respectively, with an odds ratio of 4.167 (95% CI 0,80 to 21.68, P value 0.08). AC heterozygous genotype did not show any difference between the two groups. *MTHFR* A1298C polymorphism does not associate with a higher risk, at a statistically significant level.

J05.05
Umbilical cord stem cells a boon to man-kind but karyotyping a necessity

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The isolation of umbilical cord stem cells is of great value for treatment of several disease/disorders. These cells are unique because they can be expanded to therapeutically relevant numbers and cryopreserved for different uses. Cord blood collections from full-term and babies range from 70-130 mL. Umbilical cord blood stem cells have now been used in more than 10,000 transplants as an alternative to hematopoietic stem cells. The parent's blood is tested for infectious diseases. The cord blood unit is delivered to the cord blood bank. Once stored, it is available for a transplant for the child or donated to a patient if he is a match. But many genetic disorders such as balanced translocation are not observed in the beginning or even in the whole life span. This type of umbilical cord stem cells if used in transplant can result in a severe and hazardous effects on the patient. Karyotyping is a test to examine chromosomes in a sample of cells, which can help identify genetic problems. So this type of abnormalities can be prevented from being transferred, by making karyotyping mandatory. Many parents do not consider prenatal karyotyping as it is dangerous for child so at lest it should be made mandatory during blood banking or after birth.

J05.06
Prenatal diagnosis of Klinefelter's syndrome by QF-PCR

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The 26 years old Patient has been directed for prenatal diagnosis of the most frequent chromosome aberrations Indication was the results of biochemical screening in the second trimester. We used QF-PCR to detection the most frequent chromosome aberrations. The analysis of STR markers for autosomes 13, 18, 21 (markers D13S628, D13S634, D13S742, D18S3801, D18S386, D18S391, D18S535, D21S11, D21S1437 D21S1411, D21S226,) revealed the normal parity of peaks for all analyzed loci. The analysis of molecular markers on sex chromosomes demonstrated the parity of peaks for marker AMXY - 2:1 and X22-1:2, while other markers of chromosome X (DXS981, DXS6854, P39, XHPRT) were presented by single peaks. This parity of peaks for the markers localized both on X, and on Y chromosomes (AMXY - 2:1 and X22 - 1:2) testifies for either the non-disjuncted X chromosomes in the second division of meiosis, or for the mosaic karyotype 45, X/46, XY at least in 50 % of cells. After this the Patient was recommended cordocentesis for karyotyping fetal cord blood lymphocytes. The Klinfelter's syndrome in the fetus (47,XXY) has been revealed.

To detect whether the origin of Klinfelter's syndrome in this case was due to X chromosome non-disjunction in the second division of maternal meiosis, we analyzed STR markers of X chromosome in the parental DNA samples. QF-PCR showed that extra X chromosome has maternal origin. Thus, on the basis of this case we developed the algorithm for prenatal detection of numerical sex chromosome aberrations

P06 Cancer genetics

P06.001
Investigation of expression level of MRP1 in Iranian colorectal cancer (CRC) patients

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Background: Drug resistance is still a great obstacle to the success treatment of breast cancer. The Multi Drug Resistance Related Protein1 (MRP1) is a member of the ABC-transporter that located on chromosome 16p13 and transports a wide range of compounds including glutathione conjugates and cyclic nucleotides out of cells
In this study we attempted to investigate the possible correlation between MRP1 and clinical response in patients with colorectal cancer (CRC).
Materials and Methods: Tumor and adjacent normal tissues from 60 Iranian patients with colorectal cancer were assessed for the expression level of MRP1 by Real Time RT-PCR.
Sixty CRC patients were enrolled in this study. The project was approved by the local ethical committee of National Institute for Genetic Engineering and Biotechnology (NIGEB) and written informed consent was obtained from all cases. Tissue specimens (tumor and normal tissue adjacent to tumor) were collected from Hazrat Rasool hospital between 2008and September 2010, Histological diagnosis was confirmed for all samples.
Results: No statistically significant increase in MRP1 expression level was observed when tumor tissues were compared with normal tissues. Furthermore, The MRP1 expression was also independent of the age and sex of the patients, localization and size of the primary tumor, tumor infiltration of the lymph nodes, distant metastases and histological grading.
Conclusion: Our results suggest that MRP1 unlikely have an affect the clinical response to treatment in CRC patients.

P06.002
Association between tumor necrosis factor α -308 gene polymorphism and risk of acute lymphoblastic leukemia in Serbian children

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Tumor necrosis factor TNF plays a major role in growth regulation, cell differentiation, response to viral, bacterial and other infections. TNF can be produced by tumor-infiltrating macrophages and by several types of animal and human cancers (e.g. ovarian and colorectal cancers, melanoma and hematological malignancies). Since TNF are potent controllers of cell functions, it would be of interest to demonstrate that deregulation of TNF-α production in ALL patients in genetically determined and therefore might be a contributing factor in the pathophysiology of lymphoproliferative disease.
A common genetic polymorphism in the TNF gene- G308A is associated with greater susceptibility to various carcinomas. Guanine to adenine transition at the -308 site in the TNF promoter region (TNF A allele) and LTα +250 have both been associated with variable TNFα secretion after different stimulations, with increased levels associated with the allele A, at both sites. We investigated a possible association of TNF polymorphisms G308A, and increased risk for acute lymphoblastic leukemia (ALL). PCR-RFLP analysis of the TNF gene was performed on DNA obtaind from 89 ALL patients and 124 healthy individuals.The following genotype frequencies were found: 56% (GG), 41% (GA) and 3% (AA) in ALL patients. Genotypes in controls were: 71% (GG), 29% (GA) and 0% (AA). A statistically significant difference ($p<0.05$) was found in the genotype frequencies between ALL patients and the control pointing to the association between TNF α -308 polymorphism and ALL risk in the Serbian population.

P06.003
The frequency and association of C609T and C465T polymorphisms of NAD (P) H: quinone oxidoreductase gene in adult acute myeloid leukemia

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Objective: Genetic variations and mutations are the etiological factors of leukemia. NAD (P) H: quinone oxidoreductase (NQO1) plays an important role in detoxification of quinones. C609T and C465T are two common polymorphisms in NQO1 that result in a lower NQO1 activity compared with wild type. We assessed the frequency of C609T (NQO1*2) and C465T (NQO1*3) polymorphisms of NQO1 gene among Iranian population to judge about the association between these

polymorphisms and susceptibility to adult acute myeloid leukemia (AML).
Methods: Frequencies of NQO1 gene polymorphisms were determined in 140 and 124 AML patients for NQO1*2 and NQO1*3, respectively. In addition, 80 age-sex matched controls for NQO1*2 and NQO1*3 were participated in this study. Genotyping was done using PCR-RFLP. We calculated Odd ratio (OR) and confidence interval (CI) of NQO1 genotypes to examine if these polymorphisms are associated with AML.
Results: No significant association was observed between these two polymorphisms of NQO1 and the risk of AML. Odds ratio for C609T and C465T were 0.913 (95%CI= 0.511-1.632) and 2.009 (95%CI= 0.572-7.657), respectively. Men showed a higher incidence of C609T and C465T NQO1 than women. The majority of patients with mutant allele were diagnosed as M3 sub type of FAB classification.
Conclusions: Our findings suggest that the NQO1 C609T and C465T gene variants do not have a major influence on the susceptibility to adult AML. Interestingly, we found a higher incidence of T allele in NQO1*2 than NQO1*3 in both control and patient groups.

P06.004
The effect of arsenic trioxide treatment on mitochondrial apoptotic gene expression in acute promyelocytic leukemia cell line

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Background and Objectives: Acute promyelocytic leukemia (APL) is one of the most malignant forms of acute leukemia with a fatal course of only weeks which represents 10-15% of AML in adults. Arsenic trioxide as a single agent factor (without chemotherapy) is the treatment of choice for APL patients; it induces cell death through apoptosis but the mechanism by which arsenic targets apoptosis and dramatically affects gene expression remains poorly understood. Since arsenic is used as first line treatment in Iran, it is worth investigating its effect on expression of genes involved in APL.
Materials and Methods: In this descriptive study, to understand the underlying mechanisms of cell death induction by arsenic, we treated NB4 cell line in a dose and time dependent manner. Extracting RNA and synthesis of cDNA, gene expression of apoptotic genes in mitochondrial pathway including caspase3, Mcl-1 and Bcl-2 was analyzed through Real-Time PCR.
Results: Our findings showed that As_2O_3-induced cell death was paralleled by reduced expression of the antiapoptotic protein Bcl-2 but the expression of Caspase3 and Mcl-1 did not change after arsenic treatment.
Conclusions: These results suggest that changes in Bcl-2 gene expression may be one of the mechanisms of action of arsenic in induction of apoptosis, while Caspase3 and Mcl-1 gene expression are not affected by arsenic at the transcriptional level.

P06.005
Copy number variation analysis in 134 unrelated patients with mutation negative adenomatous polyposis

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Background: In up to 50% of patients with colorectal adenomatous polyposis no germline mutation in the currently known genes - *APC* and *MUTYH* - can be identified. Copy number variants (CNVs) have recently been recognised as important forms of structural variation which also predispose to human disease. It can be hypothesised that in particular heterozygous microdeletions contribute to the underlying cause in yet unidentified genes responsible for adenomatous polyposis syndromes.
Methods: Genomic DNA from 134 unrelated mutation negative polyposis patients was used for genome-wide SNP genotyping with the HumanOmni1-Quad BeadArray (Illumina). Putative CNVs were identified by the QuantiSNP v2.2 algorithm, filtered according to various criteria by use of the CartageniaBench software, by in-silico-analysis, and by comparison with 531 healthy controls, and validated by qPCR.
Results: 35 unique heterozygous deletion CNVs containing 38 genes could be identified in 33 of 134 patients but not in healthy controls. All CNVs are present only once in the whole cohort; all except two patients harbour just one CNV. 25 genes are partly or completely deleted, in 13 more the deletion affects intronic regions only. Candidate adenoma genes include protein kinases, transcription factors, and potential tumour suppressors.
Conclusions: By applying stringent filter criteria we identified a group of rare deletions which might contain predisposing genes for adenoma formation. The further work-up will prioritize genes according to gene function and pathway. Promising candidates will be sequenced in all patients to look for germline point mutations. The study was supported by the German Cancer Aid (Deutsche Krebshilfe).

P06.006
Genetic Polymorphism of Alpha 1 Antytrypsin and Glutathione S Transferase and Lung Cancer Risk

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Background. Polymorphisms for genes encoding alpha 1 antitrypsin (A1AT) and glutathione S-transferase (GSTM1/GSTT1) might contribute to the variability in individual susceptibility to lung cancer.
Objectives. This is a cross-sectional, randomized, case control study for the evaluation of the frequency of A1AT (MS, MZ) and GST (GSTM1/GSTT1 null) alleles among patients with lung cancer.
The study included 56 cases of lung cancer diagnosed patients (histopathological examination), recruted from the Pneumology Hospital Leon Daniello Cluj and 125 healthy unrelated controls, selected among patients observed in the Internal Medicine Department.
Methods. A1AT genotyping was carried out using PCR amplification of relevant gene segment was followed by restriction enzyme digestion Taq1. Detection of A1AT gene S and Z alleles was determined through analysis of resulting restriction fragment length polymorphism (RFLP). For GSTM and GSTT genotyping we used Multiplex PCR, followed by gel electrophoresis analysis.
Results. The molecular analysis identified the MS genotype in 3 (5,4%) patients with lung cancer and 1 (0,8%) of the controls. The heterozigous MZ state was detected neither among cases nor in controls. The prevalence of GSTM null genotype in lung cancer patients was 49,4% compared to 42,8% of controls, also the prevalence of GSTT null genotype in lung cancer patients was 24,5% compared to 18,2% of controls.
Conclusions. The results of our study reached statistical significance, our findings suggest that heritable A1AT and GST status may influence the risk of lung cancer developement.

P06.007
Expression analysis of two testis specific genes OIP5 and TAF7L in peripheral blood of Acute Myeloid Leukemia patients

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Acute Myeloid Leukemia (AML) has been known as a highly heterogeneous hematological disorder which arises from a variety of alterations. Expression of testis specific genes (TSG) has been reported in various tumor types and makes a specific group of genes, named Cancer Testis Antigens (CTAs). The present study aimed to determine the expression levels of two TSG's, OIP5 and TAF7L in Peripheral Blood Mononuclear Cells (PBMCs) of AML patients and healthy subjects.
44 AML patients (subtypes: M1, M2, M3, M4, M5, M7) and 33 healthy

subjects were enrolled. Specimens were collected and PBMCs were isolated followed by total RNA extraction and cDNA synthesis. Real-time QPCR was carried out and data was analyzed by LinReg 11 and REST 2009.
Unexpectedly, our results showed dimorphic expression of these genes between affected males and females. In affected females compared to healthy females, OIP5 showed overexpression by a factor 3.079 (p<0.001) in contrast to no significant change in expression in affected males compared to healthy males. TAF7L was lower-expressed by a factor 0.162 (p<0.03) in affected males compared to healthy males versus no significant change in affected females compared to healthy females.
Overexpression of OIP5 in females could have an added advantage of analyzing its expression for MRD (Minimal Residual Disease) monitoring and also applying in immunotherapy. These findings may shed light on the different susceptibility of developing leukemia in a gender specific manner that needs to be investigated and confirmed in future.

P06.008
Androgen Receptor is a target gene for mutations in colorectal tumors with microsatellite instability and the mutated alleles undergo hypermethylation.

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Microsatellite instability (MSI) is a consequence of a functional deficiency of the mismatch repair genes, and is a feature of the majority of Lynch syndrome tumors, and of a minority of sporadic colorectal (CRC), gastric and endometrial cancers. Several genes have been described to be target for mutations of the MSI condition. Androgen Receptor (*AR*) gene has been involved in colorectal carcinogenesis.
We aimed to assess the implication of *AR* gene in the carcinogenic process of CRC with MSI.
We tested 343 sporadic CRC for MSI using five monomorphic mononucleotide markers. Polymorphic CAG repeat at exon 1 of the *AR* gene was analyzed from the normal and tumoral DNA. HpaII methylation-sensitive restriction enzyme, was used to analyze the methylation status of the CAG repeat pattern. Capillary electrophoresis and *GeneScan* analysis was used for all these experiments.
MSI was present in 31 of the 311 CRC analyzed (10%). Insertion-deletion mutations at CAG repeat were detected in 25 out of 31 MSI tumors (80.6%) and in one of the 40 analyzed microsatellite stable tumors (2.5%) indicating that *AR* might be a new target gene for MSI tumors (p<0.001). Interestingly, methylation analysis of MSI tumors with *AR* mutations showed 17 tumors with hypermethylation of the mutated alleles and two tumors without methylation (p=0.023). The remaining six cases were considered as non-informative.
Our results suggests that *AR* gene is specifically mutated in tumors with MSI and those mutated alleles might be silenced by hypermethylation. The consequences of these observations need to be explored.

P06.009
Development of a workflow to detect sequence variants in the APC Gene

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Germline mutations within the adenomatous polyposis coli (APC) gene are responsible for up to 80% of classical familial adenomatous polyposis (FAP). To date, more than 800 different APC germline mutations have been characterized. The high number and distinct spectrum of variations together with gene length make the automated capillary electrophoresis DNA sequencing the gold standard method to develop an efficient and robust analysis of this gene. We have set up a 3-step workflow to detect mutations in the APC gene in a rapid manner aimed to homogenize and facilitate the experimental work. Step 1: Analysis of 65% of exon 15 in three amplicons (which harbors the majority of mutations); Step 2: Analysis of 12 amplicons with RSA primers in a 96 well plate; Step 3: Analysis of the remaining sequence (8 amplicons). All sequencing reactions were run in a 3730XL Genetic Analyzer. We have studied 32 individuals; 8 with classical and 24 with an attenuated phenotype. Six germline variants were detected in FAP individuals and 8 in the AFAP families. Of these 7 were pathogenic and the remaining 7 were classified as USV. Ten of the 32 cases were reanalyzed on the newest 3500 Genetic Analyzer showing a perfect concordance regarding mutation detection when compared to the 3730XL analyses. Conclusion: Simple workflow adapted to distinct Genetic Analyzer Instruments is a cost-effective approach to analyze large genes such as APC. The 3730XL and 3500 Genetic Analyzer are for research use only, not for use in diagnostic procedures

P06.010
The Effects of Kaempherol and Silymarin on Cell Cycle and Apoptosis Signal Transduction Pathways at Chronic Myelogenous Leukemia Cell Line K562

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Silymarin, an active component of *Silybum marianum L.*, has been shown as a powerful antioxidant, anti-inflammatory and anticancer agent.Kaempferol, an active component of *Epilobium parviflorum*, is a flavonoid with anti- oxidant activity and protective against various cancers.
We aimed to evaluate the effects of kaempherol and silymarin in gene expression of apoptosis pathway and the expression changes of cell cycle genes in K562 cell line.
Cytotoxicity analysis was performed for silymarin and kaempherol between 4.69-300.00µM and 10.6-30.25µM doses, respectively, at 24 hours intervals for three days using XTT assay.Total RNA was isolated from the cells exposed to IC_{50} doses of silymarin and kaempherol and the expressions of 45 genes each from apoptotic pathway and cell cycle controls were studied by real time online RT-PCR.Results were compared with silymarin or kaempherol-free cells.
Silymarin, used at IC_{50} dose (29.02µM) significantly increased the expression of genes responsible for cell cycle pathway (CDK2,CDK6, CDK7,CDK8,CDKN1A,CDKN1B,CDKN2C,CDKN3,CDC25A, CDC34, CHEK1 and CHEK2) and the genes which induces apoptosis (APAF1, BCL2, BCLAF1, CASP2, CASP3, CASP4, CASP7 and CASP9).No difference was found in none of the genes following the treatment with IC_{50} doses of kaempherol (9.84µM).
In conclusion, 29.02µM of silymarin induced apoptosis and arrested cell cycle at G2/M transition in K562 cell line.These results lead to the idea that, this extract seemed to be effective in the treatment of leukemia, however further studies are needed to elucidate the molecular mechanism of these genes expressions at protein levels.
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P06.011
Towards TRAIL to silencing of SMURF and NEDD4: FLIP is flopped

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Objective: Heterogeneity of the prostate cancer offers stumbling blocks in standardization of therapeutic interventions. TRAIL and cFLIP are two diametrically opposed key mediators that antagonize each others function. In this particular study we hypothesized whether abrogation of negative regulators of TGF signaling potentiates and abolishes the expression of TRAIL and cFLIP respectively or not.
Materials and methods: we have used androgen sensitive prostate cancer cell line (LNCaP) and treated it with TGF. TGF treatment was also given to LNCaP cell line. RNA interference technique was used to unfold the correlation betweeen TGF signaling and TRAIL and cFLIP expression in the LNCaP cell line. The results were analyzed by RT PCR and western blot.
Results: We have treated the TGF treated cell line with siRNA of NEDD4 and SMURF. There was a successful blockade of both genes at transcriptional level as evidenced by RT PCR study. Simultaneously there was remarkable upregulation in the expression of TRAIL. Another interesting observation was that TGF treatment triggered expression

of TRAIL and ablated cFLIP and this activity was pronounced after abrogation of negative regulators of TGF signal transduction.
Conclusion: In this study, we show that TRAIL expression is upregulated and cFLIP is downregulated upon exposure of LNCaP cell lines to TGF-β and etoposide and that TRAIL is a major contributor to apoptosis mediated by TGF-β. it is obvious that NEDD4 and SMURF are the major proteins involved in the deviation of core biological systems and their inhibition might offer exciting avenues in translational oncology

P06.012
Aven blocks DNA damage-indused apoptosis by stabilizing Bcl-Xl

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Induction of apoptosis by DNA damaging agents involves the activation of mitochondrial apoptotic pathway. Aven has been identified as an antiapoptotic protein and has been shown to activate ATM in response to DNA damage. In this study, we demonstrated that enforced expression of Aven blocks UV-irradiation-, SN-38- or cisplatin-induced apoptosis upstream of mitochondria by stabilizing Bcl-xL protein levels in breast cancer cells. Aven silencing by RNA interference markedly enhanced apoptotic response following treatment with DNA-damaging agents. Aven is complexed with Bcl-xL in untreated breast cancer cells and treatment with DNA damaging agents led to decreased Aven/Bcl-xL interaction. Importantly, Bcl-xL was necessary for the prosurvival activity of Aven and depletion of Bcl-xL abrogated Aven-mediated protection against DNA damage-induced apoptosis. Analysis of breast cancer tissue microarrays revealed decreased Aven nuclear expression in breast
cancer tissues compared with non-neoplastic breast tissues. In particular, we detected reduced nuclear expression of Aven in infiltrating ductal carcinoma and papillary carcinoma breast cancer subtypes compared with non-neoplastic breast tissues and infiltrating lobular breast cancer tissues. Our resultssuggest that Aven is an important mediator in DNA damage-induced apoptotic signaling in breast
cancer cells and its nuclear expression is altered in breast cancer tissues, which may contribute to genomic instability in breast cancer tumors.

P06.013
Association of p53 gene variants, p21 Ser31Arg and P73 exon 2 genetic polymorphisms with the risk of bladder cancer in North Indian cohort

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Objective: TP53 is the most frequently mutated gene in all forms of human cancers. In addition to mutations, other genetic events, such as single nucleotide polymorphisms (SNPs) in the TP53 gene, have been shown to contribute to the development of various cancers. Hence we hypothesize those polymorphic sites in p53 gene variants at codon 11, Pro47Ser, codon 248 (exon 7), codon 31 of P21 and P73 exon 2 (GC/AT) may modulate risk for Bladder cancer (BC) progression in North Indians.
Materials and Method: Genotyping for these polymorphisms were done in a group of 200 BC and 200 age matched, similar ethnicity unrelated healthy controls using PCR-based methods.
Results: In P53 codon248 polymorphism, heterozygous genotype showed high risk with BC (p<0.001; OR=1.58). Whereas in case of P73 exon2, variant genotype demonstrated significant risk (p=0.014, OR=3.80), the tumorigenic effect of which was observed to be more enhanced in case of smoking exposure. Haplotype Glu-Pro-Trp/Gln was associated with two-fold risk (OR, 1.91; p=0.002). Variant genotype of P73 G4C14>A4T14 was associated with higher risk of recurrence (HR=3.04, P=0.039) in superficial BC patients receiving BCG treatment thus showing least survival (log rank= 0.029).
Conclusion: Our study provided evidence, for the first time, that the P53 codon 248 (exon7) and P73 G4C14>A4T14 polymorphisms were associated with higher risk of BC in North Indians. Also the frequency of homozygous p21 Arg/Arg genotype was too few to be meaningfully evaluated for its association with risk of BC, which warrants larger studies for further validation of our findings.

P06.014
beta2-microglobulin mutations in melanoma

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Cytotoxic CD8+ T lymphocytes can eliminate malignant cells by recognizing peptide epitopes from tumor antigens in the complex with human MHC class I molecules. One mechanism allowing tumors to escape from that control is the downregulation or complete loss of MHC class I molecules. Total loss of MHC I expression can be due to mutations in beta2-microglobulin (b2m) gene located on chromosome 15q21. The b2m protein is noncovalently associated with alpha chain of the MHC I for antigen presentation. When b2m protein is corrupted, or b2m expression is lost, stable antigen-MHC I complexes cannot be formed.
We analyzed tissue specimens of 50 primary melanomas. The tissue microdissection technique was applied to obtain DNA from the tumors. DNA from microdissected tumor tissues was obtained using phenol-chloroform method. The technique of single-strand conformation polymorphism (SSCP) was used to screen 50 melanoma samples, and 172 normal DNA samples for mutations in the b2m. The leader peptide sequence/exon 1 and exon 2 PCR products were analyzed.
Our results exclude any homozygotic/heterozygotic nucleotide change in exons 1 and 2 of b2m in 172 healthy controls. In the context of high frequency of single nucleotide polymorphisms in the human genome, b2m coding sequence is remarkably stable. To date the known exceptions are b2m mutations observed in some types of neoplastic cells. In our work, SSCP of DNA of 50 melanomas showed eight mutations in exon 1 and four mutations in exon 2. Double-stranded direct sequencing of PCR products is required to confirm these results.

P06.015
Homozygote variant genotype of AKR1C3 rs12529 polymorphism confers protection against bladder cancer

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Occupational exposure to carcinogens and cigarette smoking are among the most significant risk factors for bladder cancer. Polymorphisms in genes coding for the xenobiotic metabolizing enzymes may affect the relevant enzyme activity and therefore cause inter-individual metabolic differences. Aldo-keto reductases (AKR) are highly polymorphic phase-I drug metabolizing enzymes, which are involved in tobacco carcinogenesis. AKR 1C family member 3 (AKR1C3) is one of the four known human AKR1C enzymes. The polymorphisms in the AKR1C3 gene may lead to changes in its expression or enzyme activity. When considered together with certain demographic and environmental factors, AKR1C3 genetic polymorphisms possibly define the levels of individual susceptibility to bladder cancer.
In this study, the effect of the rs12529, a non-synonymous polymorphism of the AKR1C3 gene, on the susceptibility to bladder cancer was investigated in conjunction with cigarette smoking and sex. Using genotype data of 101 bladder cancer cases and 101 healthy controls, we demonstrated that men (OR=9,032; %95 CI=4,308-18,938) and cigarette smokers (OR=4,89; %95 CI =2,617-8,963) are under high risk for developing bladder cancer and that rs12529 homozygote variant (GG) has a protective effect (OR=0,255; %95 CI= 0,101-0,644) when compared with the wild type homozygote (CC). The protective effect of the GG genotype becomes more apparent when the odds ratio is adjusted for cigarette smoking and sex (OR=0,243; %95 CI=0,743-0,8). These results indicate a strong relationship between AKR1C3 rs12529 polymorphism and bladder cancer susceptibility.

P06.016
AKR1C3-35 A/G polymorphism in bladder cancer susceptibility

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The ten known human aldo-keto reductase (AKR) enzymes play central roles in the metabolisms of drugs, carcinogens and reactive aldehydes, which result in their activation or detoxification. They are especially involved in tobacco-related cancers, since they both activate polycyclic aromatic trans-dihydrodiols and catalyze the detoxication of nicotine-derived nitrosamino ketones. AKR1C3 is one of the four human AKR1C enzymes that are. highly polymorphic, and single nucleotide polymorphisms may confer variations in their metabolizing activities. This implies that inter-individual variations of the xenobiotic metabolism are associated with differential susceptibility for carcinogenesis. In the framework of this study, we investigated the role of the AKR1C3-35 A/G (rs1937920) polymorphism in bladder cancer formation. The genotypes of 145 cases and 81 controls were determined by PCR-RFLP method and analyzed in conjunction with their sex and cigarette smoking status, using logistic regression. A significant increase in the risk of bladder cancer was observed for the AG (OR=1,50; %95CI= 0,995-2,261) and GG (OR=1,773; %95CI=1,225-2,565) genotypes, when compared with the wild type genotype (AA). Upon adjustments for sex and smoking, the risks for both the AG and GG geotypes were further increased (OR=2,750; %95 CI=1,39-5,43 and OR=2.12; %95 CI= 1,125-4.00, respectively). According to these results, AKR1C3-35 A/G variation can be regarded as a risk factor for bladder cancer.

P06.017
Potential usage of Helix lucorum and Rapana venosa hemocyanins in bladder cancer therapy

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Urinary bladder cancer is a socially significant healthcare problem. Risk factors such as: smoking, infections, diet and aging of the world population are responsible for its growing incidence. One therapeutic approach is the usage of a nonspecific immunostimulant Keyhole limpet hemocyanin (KLH). Our laboratory investigates in vitro effect of hemocyanins derived from species Helix lucorum (HlH) and Rapana venosa (RvH), and their potential clinical use in comparison to KLH.

Three structural subunits were isolated from HlH and two from RvH. Each subunit contains 8 functional units with mass ~50 kDa.

The anti-tumor effect was investigated on 647-V bladder tumor cell line and results suggest a direct cytotoxicity mainly from functional units. Gene expression profiling of the two pathways: Human Inflammatory cytokines (PAHS011) and Signal transduction pathway (PAHS014) on the tumor cells before and after hemocyanins treatment was performed.

Results suggest upregulation of genes for IL1A, IL1B, IL8 and C3 in Group1-G1 (cell line treated with Hlh Fu-7). IL1A and IL1B were significantly overexpressed compared to Group G2 (cell line treated with RvH-II-6). Only gene for C3 showed slightly expression in G2 compared to G1.

Ten genes from the PAHS014 were highly upregulated in G1 - CSF2, CDKN1A, IL1A, VEGFA, IL8, GADD45A, ODC1, BCL2L1, FASN, and BAX. Among them: CSF2, CDKN1A, FASN and BAX had more than 10 times higher expression in group G1 versus genes in group G2.

Our result shows which genes behavior differs vastly after treatment with HIH Fu-7. They indicate the potential biological mechanism of its action.

P06.018
The prevalence of BRCA mutations in Czech patients with triple negative breast cancer

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Several studies have shown that breast tumours arising in women carrying germline mutations of the BRCA1 tumour-suppressor gene are often triple negative (negative for estrogen-receptor, progesterone-receptor and HER2 expression). In our study, we estimated the proportion of BRCA1 and BRCA2 mutation carriers among women with triple negative breast cancer.

BRCA1 screening of 109 patients who were diagnosed with triple negative breast cancer was performed in our laboratory. These patients were divided into 4 groups:

I. Patients with family history of breast/ovarian cancer or bilateral breast/ovarian cancer patients

II. Early-onset sporadic unilateral breast cancer patients diagnosed before 40 years of age

III. Patients with medullary type of breast cancer diagnosed after 40 years of age

IV. Sporadic breast cancer patients diagnosed after 40 years of age

There were identified 43 mutations (68.3%) in the first group, 6 mutations (23.1%) in the second group, one mutation (25%) in the third group and 5 mutations (15.2%) in the fourth group (overall 50,5%).

In addition there was performed BRCA2 screening of 93 patients out of group I-III with the result: four mutations (6.4%) were identified in the first group, two mutations (7.7%) in the second group and no mutation in the third group.

Our results confirm that women with triple-negative breast cancer are candidates for genetic testing of BRCA1 gene, especially those with family history. BRCA2 mutations were detectable in 6.5% of cases.

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P06.019
Evaluation of 53 sequence variants of unknown significance of the BRCA genes using bioinformatics predictions of RNA splicing and functional splicing assays

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A large fraction of sequence variants of unknown significance (VUS) of the breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2* may induce splicing defects. We analyzed 53 VUS of *BRCA1* or *BRCA2*, detected in consecutive molecular screenings, by using 5 splicing prediction programs and we classified them into two groups according to the strength of the predictions. In parallel, we tested them by using splicing minigene assays. Ten VUS were predicted by two or more programs to induce a significant reduction of splice site strength, or activation of cryptic splice sites, or generation of new splice sites. Minigene-based splicing assays confirmed 4 of these predictions. Five additional VUS, all at internal exon positions, were not predicted to induce alterations of splice sites, but revealed variable levels of exon skipping, most likely induced by the modification of exonic splicing regulatory elements. We provide new data in favor of the pathogenic nature of the variants *BRCA1* c.212+3A>G and *BRCA1* c.5194-12G>A, which induced aberrant out-of-frame mRNA forms. Moreover, the novel variant *BRCA2* c.7977-7C>G induced in frame inclusion of 6 nt from the 3' end of intron 17. The novel variants *BRCA2* c.520C>T and *BRCA2* c7992T>A induced incomplete but strong skipping of exons 7 and 18, respectively. We confirm the sensitivity of current algorithms for detecting VUS that induce changes of splice site strength or generate/activate new splice sites. However, an important fraction of internal exonic variants escape bioinformatics detection, highlighting the need of more accurate bioinformatics predictions for internal exonic variants.

P06.020

Using heteroduplex analysis by mismatch-specific endonuclease for the detection of *BRCA* mutations and SNPs

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BRCA1 and *BRCA2* are major cancer predisposition genes, responsible for a large percentage of hereditary breast and ovarian cancer (HBOC) families. Screening for mutations in these genes is now standard practice for HBOC cases in Europe, and permits medical follow-up and genetic counselling adapted to the needs of individuals in such families.

Currently, most laboratories performing diagnostic analysis of the *BRCA* genes use PCR of exons and intron-exon boundaries coupled to a pre-screening step to identify anomalous amplicons. The techniques employed for the detection of mutations and polymorphisms have evolved over time and vary in sensitivity, specificity and cost-effectiveness.

Recognising that direct sequencing is too expensive for us to apply systematically to all HBOC families for the foreseeable future, we turned to the recently developed Surveyor™ heteroduplex cleavage method as a sensitive and specific technique to reveal anomalous amplicons of the *BRCA* genes, using only basic laboratory equipment and agarose gel electrophoresis.

We managed to detect by this technique 3 *BRCA* deleterious mutations, as well as 2 unclassified sequence variants (UVs) and 3 common SNPs. All variants were confirmed by forward and reverse dideoxy sequencing. We recommend therefore heteroduplex analysis (HA) by mismatch-specific endonuclease as a sensitive pre-screening method to precede systematic sequencing. Together with other alternative techniques, HA allows us now to save about 30% of *BRCA* sequencing costs, and certainly can do even better.

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P06.021

Breast cancer genetics: Her2/neu overexpression may be a negative predictor of BRCA mutations

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BRCA-1 and BRCA-2 are the genes most frequently implicated in “monogenic” predisposition to breast cancer. Soon after clinical testing for BRCA mutations became available, a flurry of publications compared the histological characteristics and prognostic marker status of breast cancers in patients with BRCA-1 vs. BRCA-2 vs. BRCA-X (negative) patients. Invasive ductal cancer with medullary features and “triple negative” (for ER, PR and Her2/neu expression) prognostic marker status are considered suggestive of BRCA-associated breast cancer. The next wave of publications was epidemiological, whereas the current focus is molecular profiling of breast cancers for somatic mutations. In the interim, histology and prognostic markers have been displaced as major determinates in genetic risk assessment. We recently suggested that lobular histology is rarely, if at all, linked to BRCA mutations (DeLozier et. al., 2010). Our experience also suggests that her2/neu overexpression at initial diagnosis may be an important *negative* predictor of BRCA status.

We have counseled and tested 185 patients with invasive breast cancer at significant risk of carrying a BRCA mutation, for whom histology and prognostic marker information were available/adequate and for whom BRCA results were unequivocal. Of these 185 patients, 35 (19%) had Her2/neu overexpression as diagnosed by immunohistochemistry (IHC) and/or FISH assays. Twenty five patients (13.5%) were BRCA-positive, but none of the Her2/neu-positive patients had BRCA mutations, in spite of an average age at diagnosis of <40. If confirmed in larger studies, Her2/neu positivity might constitute a cost-effective means of screening prior to the offering of BRCA testing.

P06.022

Development of an easy and robust workflow to Detect Sequence Variants in the BRCA1 and BRCA2 Genes

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One of the major responsibilities of a clinical genetics research laboratory is mutation scanning in one or more genes related to an inherited disease. There are many different techniques for mutation scanning: (1) a prescreen that detects but does not characterize variants, and (2) the industry-standard technique: direct sequence analysis of the DNA by Sanger sequencing. Here we describe the development and verification of a direct resequencing workflow for mutation screening of BRCA1 and BRCA2 genes on the 3500 Genetic Analyzer .This workflow contains all steps, from purified DNA to data analysis covering both genes, PCR cleanup, cycle sequencing, electrophoresis and data analysis. To simplify workflows and decrease time to result, we focused on a robust assay based on a “one individual - one test” approach. Key to the success of this workflow was the 96-well plate design, which contained pre spotted PCR primers covering both genes and also included multiplex non template controls. Finally the use of the new developed BigDye® Direct Cycle Sequencing chemistry eliminates the need for a separate PCR clean-up step. This simplified process without a separate PCR clean-up step reduced the overall workflow time by 40%. This verified workflow permits to accelerated analysis, reduced labor time, and simplified laboratory set up enabling to skip pre screening methods. The 3500 Genetic Analyzer and BigDye® products are for research use only, and not intended for any animal or human therapeutic or diagnostic use

P06.023

BRCA1 haplotype analysis in patients with familial and non-familial breast cancer from Castilla y León (Spain)

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Pathogenic mutations in BRCA1 and BRCA2 are highly penetrating. The carriers of these mutations have a high risk, between 40 and 80%, to develop breast cancer. However, the number of women in the general population with breast cancer attributable to these mutations is very small (<5%), which is raising the possibility that there may be low-penetrance mutations in these genes that could change the susceptibility to breast cancer.

There are several mutations in BRCA1 gene that appear frequently in the population (polymorphisms). We showed more attention to the group of missense mutations in exon 11 (including c.2731 C> T [rs799917], c. 3232 A> G [rs16941] and c. 3667 A> G [rs16942] that cause changes amino acid (p. 871 P> L, p. 1038 E> G and p. 1183 E> G).

In this study, we hypothesized that clustering of mutations in exon 11 of BRCA1 gene may modify the risk of developing breast cancer in our population.

The analysis of the distribution of these polymorphisms in patients diagnosed

with breast cancer, carrying mutations in one of the BRCA genes, showed that the TGG alleles are inherited together as a haplotype.

Statistical analysis of results shows that there is a statistically significant relationship between the group of patients with familial breast cancer carriers of BRCA gene mutations and the presence of the haplotype TGG.

P06.024

Identification of novel intronic *BRCA1* variants of uncertain significance in a Thai hereditary breast cancer family

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We have screened for mutation in the entire coding sequence of the *BRCA1* gene in 50 Thai breast cancer patients. Screening for mutations was carried out using the polymerase chain reaction-based on single-stranded conformational polymorphism (PCR-SSCP) and direct DNA sequencing. The genetic alterations were not found in patients without a family history of breast cancer. One patient with a family history of five breast cancer cases harbored a germ-line mutation in the *BRCA1* gene, that was a novel intronic *BRCA1* mutation (IVS7+34_47delTTCTTTTCTTTTTT). In addition, two unclassified intronic *BRCA1* variations (IVS7+34_47delAAGAAAAGAAAAAA in the antisense strand and IVS7+50_63delTTCTTTTTTTTTTT in the sense strand) and one unclassified intronic point mutation (IVS7+38T>C) were also identified in the patient's healthy daughter at the age of 25. RNA analysis of variants of unknown significance revealed a normally occurring splicing process. These alterations were not found in family members or unrelated healthy volunteers. Of further interest, was that the deletion site was in close proximity to the triplet repeat sequence of TTC·GAA. The deletion of a set of TTC repeats was identified both in the patient and the patient's healthy daughter. It is implied that such genetic alterations could cause genomic rearrangement or an alteration to the DNA structures, possibly through a slipped strand mispairing mechanism during DNA replication.

P06.025
BRCA1 and BRCA2 germline mutations in 100 high risk Iranian breast cancer families

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Objective: Breast cancer is the most common cancer in Iranian women. Genetic predisposition accounts for 5-10% of all breast cancers and germline mutations in breast cancer susceptibility genes, *BRCA1* and *BRCA2* are responsible for a substantial proportion of high-risk breast and breast/ovarian cancer families. Therefore, the aim of this study was to investigate mutations of *BRCA1/2* in high risk Iranian families.
Methods: We screened 100 families who met our minimal criteria. The entire coding sequences and each intron/exon boundaries of *BRCA1/2* genes were screened by direct sequencing and MLPA.
Results: In the present study, we could detect the novel following mutations:
p.Gly1140Ser, p.Ile26Val, p.Leu1418X, p.Glu23Gln, p.Leu3X, p.Asn1403His, p.Asn1403Asp, p.Lys581X, p.Pro938Arg, p.Thr77Arg, p.Leu6Val, p.Arg7Cys, p.Leu15Ile, p.Ser177Thr, IVS7+83(-TT), IVS8 -70(-CATT), IVS2+9(G>C), IVS1-20(G>A), IVS1-8(A>G), p.Met1Ile, IVS2+24(A>G), IVS5-8 (A>G), IVS2(35-39)TTcctatGAT, IVS13+9 G>C in BRCA1 and p.Glu1391Gly, p. Val1852Ile, IVS6-70(T>G), 1994-1995 (InsA) in *BRCA2*.
The missense substitutions p.Leu3Stop codon, Leu1418Stop codon, p.Lys581Stop, CD26SmallINSGTCCC^ATCTG E212catctgGTAAGTCAGC,p. Ile21Val, p. Gly1738Glu, IVS2-1(G>C) in BRCA1 and p. Leu1522Phe and 1994insA 1995 in BRCA2 are pathogenic (%7).
Based on our preliminary results two haplotypes may have a pathogenic role in breast cancer development:
1. Glu23Gln, Ile26Ieu, Ile15Ieu, Arg7Cys
Gly1140Ser Leu871Pro, GLu1038Gly, Ser1613Gly, 2.
Conclusion: In agreement with findings in other populations, we found that family history is a good predictor of being a mutation carrier. Further studies are required to confirm the hypothesis that genetic polymorphisms are associated with breast cancer.

P06.026
Description of *BRCA1/2* mutations in 2.908 Spanish breast/ovarian cancer families and development of a probability model for mutation prediction

Description of BRCA1/2 *mutations in 2.908 Spanish breast/ovarian cancer families and development of a probability model for mutation prediction*
Introduction: *BRCA1* (MIM# 113705) and *BRCA2* (MIM# 600185I) germ-line mutations cause inherited susceptibility to breast/ovarian cancer (BC/OC).
Objectives: 1) To know the variety and prevalence of *BRCA1* and *BRCA2* mutations in Spanish BC/OC families. 2) To assess the association between mutation status and individual and family characteristics. 3) To estimate the probability of *BRCA1/BRCA2* mutation detection.
Patients and Methods: index cases of 2.908 BC/OC families were analyzed for *BRCA1/BRCA2* germ-line mutations, and results were correlated with individual and familiar history. A multivariate logistic regression model was fitted for either *BRCA1* or *BRCA2*. Model discrimination and calibration was measured with the area under the ROC curve and the Hosmer-Lemeshow statistic.
Results: We identified mutations in 702 families (24.5%), 355 *BRCA1* (119 different) and 347 in *BRCA2* (138 different). Proband characteristics related to mutation were: a) *BRCA1*: OC+BC (OR=7.7 and 9.6 for uni or bilatBC), OC (OR=2.8) and bilatBC (OR=2.2). Family characteristics were: OC (OR=3.5 for 1 member, OR=7.3 for $\geq$ 2 members) and bilatBC (OR=1.54 for $\geq$ 1 members). b) *BRCA2*: bilatBC and OC (OR=8.5) and male BC (OR=3.1). Family characteristics were male BC (OR=6.9 for $\geq$ 1 members), BC (OR=1.9 for $\geq$ 2), OC (OR=1.6 for $\geq$ 1 members). Ages at diagnosis were inversely associated with risk of *BRCA1/BRCA2* mutations. Areas under the ROC curve were 0.77 for *BRCA1* and 0.71 for *BRCA2*.
Conclusions: Knowledge of individual and familiar variables associated to the presence of a mutation will allow a more effective detection strategy.

P06.027
Brca2 heterozygosity promotes Kras(G12D)-driven carcinogenesis in a murine model of familial pancreatic cancer

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Inherited heterozygous BRCA2 mutations predispose carriers to tissue-specific cancers, but somatic deletion of the wild-type allele is considered essential for carcinogenesis. Using a murine model of familial pancreatic cancer that accurately recapitulates the clinical, histological and molecular features of the human disease, we find that germline heterozygosity for a pathogenic Brca2 mutation suffices to promote pancreatic ductal adenocarcinomas (PDACs) driven by Kras(G12D), irrespective of Trp53 status. Unexpectedly, tumour cells retain a functional Brca2 allele. Correspondingly, three out of four PDACs from patients inheriting the founder 999del5 mutation in BRCA2 did not exhibit loss of heterozygosity (LOH). On the other hand, three tumours from these patients with confirmed LOH were acinar carcinomas, normally a rare pancreatic neoplasm. Acinar carcinomas were also observed solely in mice bi-allelic Brca2 inactivation in the context of mutant Trp53(R270H) expression. Based on these findings we suggest a revised model for tumour suppression by BRCA2 with implications for the therapeutic strategy targeting BRCA2-mutant cancer cells.

P06.028
Predicted deleterious BRCA2 missense variants detected in Czech high-risk hereditary breast cancer families: segregation analysis and clinical data.

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In the analysis of genes associated with the cancer predisposition the deleterious mutations can be relatively easily distinguished if it is obvious that there is disruption in the coding sequence. However, rare missense and non-truncating variants found in cancer predisposing genes often create difficulties in clinical interpretation of laboratory mutation screening results.
Here we present available segregation data and clinical information

of four predicted deleterious BRCA2 missense variants located in C-terminal DNA bindings domain (exons 17 and 18) detected in Czech high-risk hereditary breast cancer families: p.His2623Arg, p.Trp2626Cys; p.Glu2663Val; Asp2723Gly; as well as two not yet described novel variants: p.Lys2630Gln and in-frame deletion p.Ala2633_Glu2635del. Other rare missense variants detected in Czech families in this region that were previously predicted or determined by functional assays as likely neutral were: p.Arg2666Thr; p.Ser2704Phe, p.Ala2717Ser; p.Val2728Ile and p.Lys2729Asn. None of all these BRCA2 variants was detected in 100 unrelated elderly (>60 years of age) healthy Czech women without any personal history of cancer and without family history of breast, ovarian, pancreas or colorectal cancers.
Segregation analysis in hereditary breast cancer families provides information to distinguish between deleterious and neutral missense alterations identified in BRCA2 gene, but most mutations are very rare and familial data are often insufficient. There is an enormous need to have a reliable functional assay. A novel functional assay using a syngeneic knock-in cancer cell line model is going to be applied to some of these variants.
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P06.029
Search for novel germ-line mutations in familial BRCA1/2-negative breast cancers

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Major breast cancer (BC) genes, BRCA1 and BRCA2, contribute to no more than 20-25% familial BC clustering; therefore the majority of BC-predisposing mutations remain to be identified. We have selected 95 BC cases, which showed accumulation of clinical signs of the genetic disease (e.g., occurrence of BC among multiple relatives and/or bilaterality and/or young age), but could not be explained by BRCA1/BRCA2 alterations or recurrent mutations in CHEK2 (c.1100delC, c.444+1G>A, del5395) or NBN (c.657_661delACAAA) genes. The analysis of coding regions was performed using HRM (high resolution melting) followed by DNA sequencing. First, we considered the extended study for the CHEK2 and NBN: these genes are well known for the founder BC-predisposing mutations, but, surprisingly, they have not been subjected to a systematic full-length screen. However, no novel mutations in CHEK2 or NBN have been detected in our series. Next, we investigated the PALB2, BRIP1, BARD1, and RAD51C. These genes were shown to cause familial BC in selected publications, but their global-wide relevance remains to be evaluated. We have identified 2 instances of the PALB2 mutation (p.R414X, p.Q921X) in bilateral BC cases. We then continued the analysis of other genes involved in double-strand break DNA repair; by the time of abstract submission, the CHEK1, PARP1, PARP2, ERCC1, XPE, BLM, BRD7, RNF8, RAD51A, and FANCG have been investigated. One BC case contained insertion/deletion mutation in the FANCG gene (c.520_524delTCTAinsC). We suggest that HRM/sequencing analysis of candidate genes in BRCA1/2-negative familial BC cases is a realistic approach to pinpoint new hereditary cancer mutations.

P06.030
Evaluation of *RAD51C* as a new breast cancer susceptibility gene in the Belgian/Dutch population

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Recently, germline mutations in *RAD51C* were found to be associated with an increased risk for breast and ovarian cancer. Meindl et al. (2010) detected six monoallelic pathogenic mutations in *RAD51C* by screening 1.100 unrelated German women with gynaecologic malignancies (breast and/or ovarian tumors). Strikingly, all six deleterious mutations were exclusively found within 480 *BRCA1/2* negative breast and ovarian cancer families and not in breast cancer only families.
With this study we aim to determine the prevalence of germline *RAD51C* mutations in Belgian/Dutch breast and ovarian cancer families, previously found to be negative for *BRCA1&2* mutations. We performed mutational analysis in 350 index patients. Mutation detection was performed with High resolution melting curve analysis (HRMCA), followed by Sanger sequencing of the aberrant melting curves.
Besides frequent single nucleotide polymorphisms and some novel, rare variants of which the clinical significance is currently under evaluation, we did not identify any deleterious mutation. To increase the number of probands to a similar number as in the initial report, we screened 100 additional families with HRMCA. Sequencing analysis of the fragments with aberrant melting curves is currently ongoing. We are the first group to investigate a cohort of breast and ovarian cancer families of comparable size as Meindl et al., and based on their findings, we had expected to detect at least three deleterious mutations. As other studies on (male) breast cancer families also did not identify deleterious germline *RAD51C* mutations, these may be less frequent than initially reported.

P06.031
Germ-line mutations and sequence variants of breast cancer susceptibility genes BRCA-1 and BRCA-2 in a Sri Lankan cohort of breast cancer patients

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A cohort of familial (N=66 for BRCA-1; N=55 for BRCA-2) and sporadic (N=64 for BRCA-1 and N=54 for BRCA-2) breast cancer patients and at risk individuals (those with a positive family history of breast cancer but not yet affected by the disease; N=70 for BRCA-1 and N=20 for BRCA-2) were studied to identify possible mutations and sequence variants in the breast cancer susceptibility gene *BRCA-1* and *BRCA-2* . DNA extracted from peripheral venous blood samples was amplified by polymerase chain reaction using target specific primers and the PCR products were analysed using single strand conformation polymorphism and DNA sequencing.
Nineteen sequence variants including six previously unreported variants were identified in the *BRCA1* gene and twenty three sequence variants including ten previously unreported variants were identified in the BRCA2 gene. Novel *BRCA-1* sequence variants included two deleterious frame-shift insertion mutations, c.3086delT/exon11 and c.5404delG/exon21. Novel *BRCA-2* sequence variants included two deleterious frame-shift mutations, 2403 insA / exon 11 and 2667 insT / exon 11, two possibly pathogenic mutations, 1191 A>C /exon 10, 5695 A>C /exon 10 and one unclassified intronic variant IVS15-21 insTT.
Prevalence rate of pathogenic and possibly pathogenic mutations in familial breast cancer was 6.25% for *BRCA-1* and 12.73% for *BRCA-2.* Of these, one pathogenic mutation (c.2403insA in exon 11) was seen in four unrelated breast cancer patients. Thus screening for *BRCA-2* mutations, in particular for 2403 insA / exon 11 is likely to be useful in Sri Lankan clinical practice.
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P06.032
Large genomic aberrations in BRCA1 and BRCA2 genes in Bulgarian breast cancer patients

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Background: Studies on different population worldwide have shown that the large-fragment aberration in BRCA cancer susceptible genes account for the large amount of hereditary breast and ovarian cancer cases.
Material and methods: In the present study we performed MLPA analysis of the large genomic BRCA1 and BRCA2 alterations in 100 Bulgarian breast cancer patients fulfilling the BCLC criteria. MLPA was carried out by SALSA MLPA KIT P002 -C1 BRCA2 and SALSA MLPA KIT P090 BRCA2 (MRC-Holland). The results analyzed by Coffalyser data analysis software.
Results and discussion: The frequency of the large genomic BRCA1/2 alterations found in the present study was 8%, which was in consistency with previous studies in other populations. Four genomic alterations were found in the *BRCA1* gene (4%): del *BRCA1* ex 11A in patient with both breast and ovarian cancer; del *BRCA1* ex 13 in a women with early onset and family history of breast cancer; dup *BRCA1* ex 14-15 and dup *BRCA1* ex 20 in two patients with family history of breast cancer. The observed *BRCA2* mutations were also four (4%): del *BRCA2* ex 3 in a patient with family history of breast cancer; del *BRCA2* ex 25-27 in a young women with early onset and family history; dup *BRCA2* ex 9 and dup *BRCA2* ex 27, respectively, in two women with early onset and family history of breast cancer.
Conclusion: Combination of MLPA with the conventional methods for mutation screening will assist the discovery of all spectra of mutations in Bulgarian breast cancer patients.

P06.033
Mutation screening of *BRCA2* exons 11 in Bulgarian breast cancer patients

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Background: Studies on different populations worldwide demonstrate that germ line mutations in *BRCA* genes account for the majority of hereditary breast and ovarian cancers.
Materials and methods: We have screened 130 breast cancer patients, fulfilling the BCLC criteria, for germ-line mutations in BRCA2 exon 11. Mutation analysis was performed by direct sequencing using 17 primer pairs.
Results: The founder mutation described in Ashkenazi Jews: 6174delT was observed in one patient with bilateral breast and ovarian cancer. The mutation was also found in the healthy daughter of the patient. Another deleterious mutation 5854delAGTT was detected in two patients with family history of breast cancer. In addition we found one rare missense alteration in codon 1915(C>T) leading to replacement of Thr with Met in three patients - one with early onset and two with strong family history of the disease. *Conclusions:* Protein-truncating mutations of BRCA2 are usually deleterious and increase the risk of breast cancer up to 80% over a lifetime. The mutations discovered in the studied Bulgarian breast cancer patients were previously observed in other European populations. It has been known that 6174delT founder mutation occurs very frequently (1.5%) among Ashkenazi Jews, but also appears in non-Jewish individuals from Europe, Canada etc. The Thr1915Met polymorphism (European population diversity C/T 6.4%) is a common variant in Poland that increases the risk of DCIS with micro-invasion. Further analysis of the other BRCA2 exons is necessary in order to ascertain the full spectra of the BRCA2 mutations in the Bulgarian breast cancer patients.

P06.034
The Risk of Breast Cancer in Women with a CHEK2 Mutation with and without a Family History of Breast Cancer

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PURPOSE: To estimate the risk of breast cancer in a woman who has a CHEK2 mutation depending on her family history of breast cancer. To evaluate whether CHEK2 mutation testing may be useful in clinical practice.
PATIENTS AND METHODS: 7,494 BRCA1-mutation negative breast cancer patients and 7,217 controls were genotyped for four founder mutations in CHEK2 (del5395, IVS2+1G>A, 1100delC, I157T). Odds ratios were constructed for the cases, subdivided by the number of breast cancer cases reported in first-and second-degree relatives.
RESULTS: A truncating mutation (IVS2+1G>A or 1100delC or del5395) was present in 227 (3.0%) cases and in 70 (1.0%) controls (OR = 3.0; 95% CI = 2.4 to 4.2). The OR was higher for women with a first- or second-degree relative with breast cancer (OR = 4.4; 95% CI = 3.1 - 6.2) than for women with no family history (OR = 2.9; 95%CI = 2.2 - 3.8). If both a first- and second-degree relative was affected with breast cancer, the odds ratio was 6.4 (95% CI 2.9 - 14.3). Based on the observed odds ratios, and assuming a baseline risk of 6%, we estimate the lifetime risks for carriers of CHEK2 truncating mutations to be 25% for a woman with one second-degree-relative affected, 30% for a woman with one first-degree relative affected, and 38% for a woman with both first- and one-second degree relative affected.
CONCLUSION: CHEK2 mutation screening detects a clinically meaningful risk of breast cancer.

P06.035
Development of a new method in BRCA1 and BRCA2 genetic analysis based in CSGE

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Breast cancer is the most frequent cause of cancer in women. Inherited breast cancer tend to occur earlier in life than sporadic cases and are more likely to involve both breasts. The BRCA1 protein acting as a gatekeeper in maintaining genome integrity. Therefore, any loss of function in BRCA1 protein will result in accumulation of genetic defects leading to cancer. Mutations in BRCA1 and BRCA2 are inherited in an autosomal dominant pattern. Each offspring of an individual with a BRCA1 or BRCA2 cancer-predisposing mutation has a 50% chance of inheriting the mutation. Mutation detection is thus important to detect anu susceptibility in family members. Molecular genetic testing of asymptomatic family members at risk of inheriting either a BRCA1 or BRCA2 cancer-predisposing mutation is possible once the family-specific mutation has been identified.
In this paper we present two families with new mutation in BRCA1 and BRCA2, both of them have family history of breast cancer.
To detect mutations in all exons we developed a new method based on CSGE(conformation sensitive gel electrophoresis) this new method permitted us to examine all exons and important intronic regions of BRCA1&2 will minimum of expense and with high sensitivity. PCR products that show heteroduplex pattern on CSGE gel, were subjected to direct DNA sequencing to identify the causative mutation. This molecular genetic test have shown mutation in BRCA2 gene (IVS 10+12 delT) in the first proband. The other one has a transition mutation (C to T) that results in a stop codon in codon 934.

P06.036
Aberrant promoter hypermethylation of Dickkopf-1 (DKK1) gene in tumor and adjacent normal tissue in breast cancer patients.

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Breast cancer is the most common malignancy in women worldwide. Dickkopf-1 (DKK1) gene product is a potent inhibitor of Wnt pathway. In this study, DKK1 methylation status and its protein expression were examined in normal and cancer tissues of same patients.
DKK1 promoter methylation was assessed by bisulfite-converted-MSP technique in breast cancer patients. We examined methylation changes in 83 tumors and adjacent normal tissues and also in 6 out of 9 normal breast tissues from unaffected women (no breast cancer history) as controls. Immunohistochemical staining was used to investigate DKK1 protein expression in 9 controls and 70 out of 83 tumors as well as adjacent normal tissues. Immunohistochemical result was categorized as positive or negative for DKK1.

DKK1 gene methylation was detected in normal breast tissue from unaffected women (33%). DKK1 gene was highly methylated in tumors (70%) and adjacent normal tissues (61%). Immunohistochemically, 67% normal breast tissue from unaffected women, 69% tumors and 83% adjacent normal tissues were DKK1 negative. DKK1 promoter methylation and protein expression were not associated with age, tumor size, lymph node status, histological grade, and oestrogen receptor or progesteron receptor or CERB2 positivity. Our results showed that there is a positive correlation between hypermethylation pattern and lower expression level. However, there were no differences DKK1 promotor methylation and protein expression levels between tumor and adjacent tissues. This result indicates that there is no different methylation pattern in either breast tumor or normal breast tissue, indicating that DKK1 is not specific for breast tumor.

P06.037
Differential allelic expression of BRCA1 and BRCA2 in normal cells does not drive specificity of allelic imbalance in tumors of breast cancer patients

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Differential allelic expression (DAE) is a phenomenon in which the two alleles of a gene are not expressed at equal levels. DAE affects a substantial fraction of the human genome, and has been suggested to be an important cause for human phenotypic variability, including complex traits and diseases. We have explored the hypothesis that DAE at *BRCA1* or *BRCA2* may play a role in the pathogenesis of breast cancer. Both *BRCA1* and *BRCA2* are tumor suppressor genes; breast tumors developing in women who have inherited an inactivating mutation in either of these genes have virtually always lost the wild type allele at the gene locus. In a series of 130 breast cancer cases, we determined allele-specific expression levels of *BRCA1* and *BRCA2* in normal skin fibroblasts cultured from mastectomy tissue. DAE was detected in 23% of patients at *BRCA1*, and 38% of patients at *BRCA2*. We then analyzed the allelic status of both genes in genomic DNA isolated from matching tumor samples. Allelic imbalance was detected in 73% and 52% of tumors at *BRCA1* and *BRCA2*, respectively. DAE at *BRCA1* in normal fibroblasts was not correlated with allelic imbalance at the *BRCA1* gene locus in the tumor, nor did we observe a preferential loss in the tumor of the allele that was expressed at highest level in corresponding normal tissue. A similar finding was made for *BRCA2*. We conclude that DAE at *BRCA1* and *BRCA2* does not operate in conjunction with allelic imbalance to contribute to tumorigenesis in the breast.

P06.038
Investigating FGFR2 and B7-H4 gene polymorphisms and their effects in breast cancer

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FGFR2 is amplified and overexpressed in breast cancer. In recent years, some publications have reported the role of FGFR2 polymorphisms in breast cancer risk. B7-H4 is reported to be highly expressed in almost all breast ductal and lobular carcinomas. B7-H4 overexpression seems to occur early in breast carcinogenesis. In a study performed, an association was detected between B7-H4 polymorphisms and breast cancer risk.
In our study, FGFR2 gene rs1219648, rs2981582 SNPs were studied by sequence analysis and B7-H4 gene rs10754339, rs10801935, rs3738414 SNPs were studied by PCR-RFLP method in 31 cases with breast cancer and in 30 women control.
Although statistically not important, the frequency of FGFR2 rs1219648, rs2981582 heterozygous polymorphisms and B7-H4 rs10801935 polymorphism AA, AG genotypes were found to be higher in breast cancer cases. In contrast to the previously found, B7-H4 rs3738414 polymorphism GG genotype frequency was found to be higher in the control group (p=0,018).
According to the literature scan it seems that our study is the first study results evaluating FGFR2 and B7-H4 gene polymorphisms together in breast cancer, frequency of these polymorphisms and their relations with breast cancer should be revealed with studies including large case numbers.

P06.039
The influence of MDR1 gene C3435T, T1236C, G2677T/A, A2956G polymorphisms on breast cancer development risk

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The P- glycoprotein (P-gp), encoded by the MDR1 (ABCB1) gene, is a member of the family of ATP binding cassette (ABC) transporter proteins. P-gp, which needs ATP energy to be functional, prevents the accumulation of potentially toxic substances and metabolites by extruding them out of the cell. The single nucleotide polymorphisms (SNPs) of the MDR1 gene can cause differences in P-gp expression levels and this is associated with predisposition to some diseases including breast cancer. In the current study MDR1 gene polymorphisms [C3435T (rs1045642), T1236C (rs1128503), G2677T/A (rs2032582), A2956G] were identified by Polymerase Chain Reaction - Restriction Fragment Length Polymorphism Method (PCR - RFLP) in 30 cases with breast cancer and 20 women control. When the patient group and healthy controls were compared, the only significant difference was observed in the C3435T polymorphism. TT genotype frequency was higher in the patient group compared to the healthy controls (p= 0.047) and CT genotype frequency was higher in the control group compared to the patient group (p= 0.08). According to our results TT carriers may have an increased risk for developing breast cancer. However more studies with large case numbers must be carried out in order to determine the role of MDR1 gene SNPs in the risk of breast cancer development.

P06.040
Exome sequencing of 12 familial breast cancer cases without a mutation in *BRCA1* or *BRCA2*

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The large majority of families with multiple cases of breast cancer can not be linked to mutations in *BRCA1* or *BRCA2*. In this study, we investigate the possibility that a proportion of the non-*BRCA1/2* families is due to rare high-risk genes.
To reduce the genetic heterogeneity, we selected six non-*BRCA1/2* families in which the breast cancers of most patients shared a particular array CGH profile. By means of next generation sequencing, we performed exome sequencing on germline DNA of two family members affected with breast cancer per family. Only variants that were shared between the family members were considered. Intergenic, intronic or synonymous coding variants, together with homozygous variants were excluded. Variants present in dbSNP or 1000 genomes were excluded if the allele frequency in any population was higher than 1%.
The selected families collectively showed a linkage peak on chromosome 4 with a homogeneity LOD score of 2,49. When focusing on this region, there were no genes in which breast cancer patients of all six families had a variant.
At the moment of abstract submission, we are following up on genes having variants in two or more families and are extending the data analysis to genes outside the chromosome 4 region.

P06.041
Silencing of a ubiquitin specific protease; USP32 in HeLa and MCF7 cancer cell lines

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Previous studies identified chromosomal band 17q23 as a frequent site of gene amplification in breast cancer. *USP32* is one of the oncogene candidates in this region. Ubiquitination changes activity, localization, half-life, conformation, and stability of proteins. Hence, ubiquitination and deubiquitination may have important consequences in cells. Previously, USP32 was shown to be an active deubiquitinating enzyme by an *in vivo* deubiquitination assay in our laboratory. We hypothesized that amplification of *USP32* may contribute to breast tumorigenesis by its deubiquitinating activity. Real time RT-PCR was performed to detect *USP32* overexpression in breast cancer cell lines and primary tumors. Our results showed that *USP32* was expressed more than 5-folds in 30% of human breast cancer cell lines and 2-folds in 22% of human primary breast tumors.

RNAi silencing of *USP32* was achieved by pSUPER shRNA and confirmed by real time RT-PCR. Proliferation rate of *USP32* silenced cells was determined by the MTT Assay. Transwell migration assay was used to compare migration abilities of *USP32* silenced cells. Stable silencing of *USP32* in HeLa and MCF7 cells caused a decrease in the proliferation rate up to 35%. Migration abilities of HeLa and MCF7 cells were decreased after *USP32* silencing as tested by transwell migration assay. In short, we detected that *USP32* had roles in cell proliferation and migration which may be important for understanding the complex mechanisms of breast tumorigenesis. We are conducting expression experiments in non-tumorigenic breast cell line MCF10A to further delineate its function in cells and contribution to tumorigenesis.

P06.042
IDH1, IDH2 and TP53 mutations in Bulgarian patients with glial tumours

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Glioma is the most common primary brain cancer and it is characterized with high mortality. A recent analysis of gliomas has revealed somatic mutations in the *IDH1* and *IDH2* genes, which encode cytosolic and mitochondrial NADPH-dependent isocitrate dehydrogenase, respectively. Genetic aberrations in *IDH1* or *IDH2* genes have been commonly found together with mutated *TP53* gene and associated with good prognosis for the patients.

Materials and methods:

To validate prognostic significance of genes *IDH1*, *IDH2* and *TP53* in Bulgarian patients, we screened 50 primary intracranial tumours, including astrocytomas and oligodendrogliomas. All samples were examined by direct sequencing of exon 4 of *IDH1* and *IDH2* genes, and exon 5-8 of *TP53* gene.

Results:

IDH1 mutations were detected in 12 (24%) gliomas, all were G>A changes within the isocitrate binding site (R132) at position 395. No mutation was found in *IDH2*. Aberrations in *TP53* were detected in 12 (24%) tumours. Five (42%) of them carried also an *IDH1* mutation. Patients with mutations in both *IDH1* and *TP53* were younger (median age 35) then those with an aberration only in one gene (49.5) or non-mutated cases (55; p=0.016). Median survival of patients harbouring mutations in two genes was 44.3 months compared to those with mutation in one of the genes and non-mutated cases - 11.9 and 7.3 months, respectively (p=0.04).

Conclusions:

IDH1 and *TP53* mutations are common in gliomas with good survival and may be used as specific prognostic biomarkers in Bulgarian patients with brain tumours.

P06.043
The expression of multiple cancer-testis antigens in cancerous cell lines

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Cancer-testis antigens (CTAs) are a gene family which only express in testis tissue, but some of them are randomly expressed in some types of cancers. Since testis is an immune privileged site, if these genes are expressed in tumors, they can be immunogenic. Therefore, these antigens are promising targets for cancer-specific immunotherapy. We studied the expression of NY-ESO-1 1a, NY-ESO-1 1b, SCP-1, SSX-2 and MAGE-3 in different cell lines (A375 , U373MG , 1321N1 , RPMI , MCF , MDAM13 453 and PC3) by Multiplex RT-PCR method.

Total RNA was extracted from the cell lines and cDNA synthesized, after wards Multiplex PCR performed by using primers of each CTA gene and GAPDH as internal control. The expression of these genes was detected with gel electrophoresis and staining with ethidium bromide (the results are presented in Table-1).

This preliminary study is in agreement with previous data on expression of CTAs in tumors and the potential use in immunotherapy of cancers.

Table 1; The results of expression of CTAs in seven diferent cell lines. N.D=Not Determind

Cell Line	NCBI Code of Cell Line	NY-ESO-1 1a	NY-ESO-1 1b	MAGE-3	SCP-1	SSX-2	GAPDH
A-375	C136	+	+	+	-	-	+
U-373 MG	C455	-	-	+	-	-	+
1321N1	C118	N.D	+	N.D	+	-	+
RPMI 8866	C210	N.D	-	N.D	-	-	+
MCF-7	C135	-	-	-	-	-	+
MDA-MB-453	C214	-	-	-	-	-	+
PC-3	C427	N.D	-	N.D	-	-	+

P06.044
Canine mammary tumours are excellent models for human breast cancer

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Breast cancer is a major contributor to overall morbidity and mortality in women and the genetic causes underlying the disease are unclear in the majority of the cases. Canine mammary tumours (CMT) are suitable naturally occurring tumour models for human breast cancer and share many similarities, including epidemiological, clinical, morphological and prognostic features. The knowledge about the inherited risk factors underlying CMT is limited, but clear breed predispositions exist, with 36% of English Springer Spaniels (ESS) in Sweden being affected.

We have collected a large material of ESS samples with in-depth clinical information about each dog. Ten human breast cancer genes (*BRCA1*, *BRCA2*, *CHEK2*, *ERBB2*, *FGFR2*, *LSP1*, *MAP3K1*, *RCAS1*, *TOX3* and *TP53;* 4-9 SNPs per gene) have been evaluated for association with canine mammary tumours ESS female dogs, 212 CMT cases and 143 controls. The two tumour suppressor genes *BRCA1* and *BRCA2* were significantly associated with CMT (p_{Bonf}=0.005, and p_{Bonf}=0.0001 respectively). Both *BRCA1* and *BRCA2* confer a ~4 fold increased risk for CMT. Preliminary genome-wide association results from the ESS cohort suggest that at least ten loci contribute to the disease and several associated regions contain plausible candidate genes. Further work includes targeted resequencing of candidate loci as well as DNA and RNA tumour/normal comparisons.

Our results suggest that CMT is an excellent model for human breast cancer, indicating that both humans and dogs can benefit from further comparative studies. This could provide a unique opportunity to

improve prevention, diagnosis and treatment of both CMT and human breast cancer.

P06.045
Analysis of CD38 and ZAP70 mRNA expression within cytogenetic subgroups of chronic lymphocytic leukemia in Iranians

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Chromosomal abnormalities, CD38 and ZAP70 expression profile are major independent prognostic markers in B-cell chronic lymphocytic leukemia (B-CLL). The present study was designed to investigate possible correlation between these three markers. CD38 was determined using flowcytometery in 65 patients' blood and ZAP70 expression using Real Time RT-PCR was examined in 20 B-CLL patients with del13q14, 13 patients with del11q22, 15 patients with trisomy 12 and 16 patients with undetected chromosomal abnormalities. CD38 shows weak prognostic value and also weak correlation with ZAP70 expression. Molecular analysis revealed that ZAP70 expression in del13q subgroup was the same as control group whilst it increased 2.7838 fold in del11q subgroup and 2.9547 fold in trisomy 12 subgroups compared to 15 cases of control group. Comparison of the mean and Standard Deviation of the ZAP70 expression profile within the subgroups showed that its value between the individuals of the del11q and trisomy 12 subgroups were highly variable, as against tight clustering for del13q subgroup. Therefore, there is a correlation between del13q aberration which has good prognosis with normal level of ZAP70 expression, whereas due to wide variation, no conformity is seen for del11q and trisomy 12 subgroups as poor prognostic discriminators.
It could be concluded that CD38 could not be regarded as a reliable prognostic factor, and has a poor concordance with ZAP70 expression profile whereas ZAP70 shows a good concordance with cytogenetic abnormalities and simultaneous analyzing of these two markers produce better prognosis.

P06.046
CHEK2 contributes to hereditary susceptibility to breast cancer in non-BRCA families

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Mutations in the *BRCA1* and *BRCA2* genes are responsible for only a part of hereditary breast cancer (HBC). The origins of "non *BRCA*" HBC families may be attributed in part to mutations in genes giving moderate risk, such as *CHEK2*. We investigated the contribution of *CHEK2* mutations to non-*BRCA* HBC by direct sequencing of its entire coding sequence. Fifteen mutations were discovered among 507 non-*BRCA* HBC cases and four among 513 controls. The frequency of *CHEK2* variants was significantly higher among cases (p= 0.0076), and gave an OR for breast cancer of 4.72 for deleterious mutation carriers. We then used both in silico tools and in vitro kinase activity to evaluate recombinant mutant proteins. Tumor characteristics and tumor grade of paraffin-embedded tissue blocks from 8 *CHEK2* mutated patients were evaluated by histology. To further caraterize those tumors, breast cancer immunohistochemical markers such as hormone receptors, HER2 and P53 were assessed. Because the mechanisms of tumorigenesis in association with *CHEK2* variants are still unclear, we performed genetic and epigenetic analysis of those tumors. Three revelant SNPs spanning the *CHEK2* gene locus were used to determine loss of hetherozygosity (LOH). Also, the proximal CpG islands of the *CHEK2* gene were investigated for hypermethylation. Our results suggests a contribution of *CHEK2* mutations to non-*BRCA* HBC, though the usefulness of moderate penetrance genes for genetic counseling remains controversial.

P06.047
The analysis of NPM1, FLT3 and c-KIT gene mutations in childhood acute myeloid leukemia in Russia.

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Acute myeloid leukemia (AML) patients without chromosomal abnormalities are classified into intermediate risk group; however they have various prognosis and clinical outcome. To estimate new prognostic and diagnostic markers we studied the frequencies of NPM1, FLT3 and c-KIT genes mutations in childhood AML in Russian population. 188 de novo AML paediatric patients with the median age of 8 have been studied. In parallel, samples were analyzed for most common AML chromosomal aberrations using diagnostic biochip, and the aberrations were found in 76 (40.4%) of 188 AML patients. The NPM1 mutations appeared in patients without cytogenetic aberrations only. The frequency was 3.7% (7/188) of total AML patients group and 6.2% (7/112) of patients without cytogenetic aberrations. The FLT3 mutation appeared in 10.5% (21/188) of all AML patients, in 11.6% (13/112) of patients without cytogenetic aberrations and in 10.5% (8/76) of patients with aberrations. The FLT3/ITD and FLT3/TKD were found in 7.9% (15/188) and in 3.2% (6/188) of all AML patients, correspondingly. The c-KIT mutations were found in 2.7% (5/188) of all AML patients. The mutation in c-KIT exon 17 was found in 0.5% (1/188) of all AML patients. Different mutations in c-KIT exon 8 were found in 2.1% (4/188) of AML cases. Since the mutations in NPM1 gene are significantly associated with normal karyotype (P<0.05) it makes them convenient diagnostic markers for MRD monitoring. The biochip for the analysis of mutations mentioned may be useful for clinical practice.
The work was supported by Russian Foundation for Basic Research (project no. 08-04-01480).

P06.048
Comparison between two molecular methods for the monitoring of post-transplant chimerism

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Hematopoietic stem cell transplantation (HSCT) is the gold standard treatment in a multitude of malignant and non-malignant disorders, like severe aplastic anemia, acute and chronic leukaemia and lymphomas. In the early post-transplant period after allogeneic HSCT, coexistence of the host and donor lympho- and hematopoietic systems will develop. This period, which is temporary in the majority of successful stem cell transplants, is referred to as mixed chimerism, whereas complete chimerism denotes the situation when all cell lineages are reconstituted by donor-derived cells. Successful engraftment correlates with the engraftment of donor cells and relapse is thought to evolve from occult autologous malignant cells that survive to the pretransplant marrow-ablative conditioning regimen and escape surveillance by allogeneic immune effector cells. Early analysis of donor and recipient chimerism patterns after HSCT gains importance in predicting engraftment and rejection as well as in persistent and recurrent disease. It is also helpful in guiding risk-adapted immunotherapy for prophylaxis and interventional therapy of graft-versus-host disease (GvHD) or imminent relapse.
Several techniques have been reported for this purpose. The intent of the present study is to test a novel approach for the quantification of mixed chimerism using a Indel- marker RT-PCR methode and to compare this technique to the gold standard one, i.e. STR-PCR, to assess the sensibility of RT-PCR in the analysis of chimerism and its ability to predict relapse.

P06.049
Dysregulation of apoptotic pathways in chordoma involving FAS and FASL: implications on notochord regression in zebrafish model

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Chordoma is a rare malignant bone tumor arising from notochord remnants, characterized by local invasiveness and variable tendency for recurrency. Given the implication of apoptosis in notochord regression, we studied the expression of 8 proapoptotic genes mapping in 1p36 region by RT-PCR in 32 tumours showing loss of heterozigosity (LOH) in 83% of cases. None of the analyzed tumour expression profiles overlaps with that of the control and the absence of 1p36 LOH has been correlated to a better prognosis. As the apoptotic pathway mediated by FAS-FASL was found to be involved in notochord regression, we studied their expression in 34 chordomas and observed that most of them express FAS, but not FASL. To verify a possible implication of FAS/FASL pathway dysfunction during tumorigenesis, we started *in vivo* studies on zebrafish. We investigated *zfas/zfasl* expression in embryos' total RNA during different developmental stages and observed that, while *zfas* has maternal and ubiquitous zygotic expression, *zfasl* has maternal and specific developmental stages expression. Notochordal cells were then sorted by FACS, following the injection of a GFP construct activated by a specific physaliphorous cell promoter, to study z*fas/zfasl* expression. While *zfas* expression was detected at both 24hpf and 48hpf, *zfasl* was not found expressed; further developmental stages will be studied to define the *zfas/zfasl* expression profiles. Further analyses are in progress to address functional studies aimed at opportunely silencing *zfas/zfasl*. Aberrant phenotypes during development will be analyzed verifying whether the reactivation of *zfas/zfasl* pathway might rescue normal phenotype.

P06.050
The effect of silymarin and kaempferol on the expression levels of microRNAs

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MicroRNAs (miRNA), single strand, non-coding RNA molecules with the length of 18-25 nucleotides, play a major role in the regulation of gene expression. Mature miRNA molecules are partially complementary to one or more tumor suppressor or oncogenic messenger RNAs (mRNA) and cause mRNA degradation or inhibition of the protein translation.
Silymarin is an active component of Silybum marianum L. and has been shown as a powerful antioxidant, anti-inflammatory and anticancer agent. Kaempferol, a flavonoid, is an active component of Epilobium parviflorum and presents anti- and pro-oxidant activity in various cancers.
In this study, it is aimed to investigate miRNA expression differences in the Chronic Myeloid Leukemia cell line, K562, following the treatment with Silymarin and Kaempferol compounds.
Cytotoxicity analysis was performed with several doses of silymarin (between 4.69 and 300 µM) and kaempherol (between 6 and 30µM) at 24 hours intervals for three days in K562 cell line, using XTT assay. miRNAs were isolated from cells exposed to IC50 doses of silymarin and kaempherol. Relative quantitation of 88 miRNAs was measured by real time online RT-PCR. Alterations in the miRNA expression were compared with kaempherol or silymarin- free K562 cells.
It is found that, IC50 doses of silymarin (29.02 µM) and kaempherol (9.84 µM) inhibited oncogenic miR-21, miR-128 and miR-15b and increased the expression levels of miR-22, miR-29a and miR-29c, which are reported to can function as tumor suppressors. Studies on the effects of selected plant extracts on miRNA expression profiles are may bring new clinical approaches for therapy.

P06.051
Cisplatin induces apoptosis in MCF-7 and T47D breast cancer cell lines via up regulation of bax gene and down regulation of bcl-2 gene

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Cancer cell apoptosis can be induced by Cis-diamminedichloroplatinum II (cisplatin), an efficient anticancer agent. Bcl-2 and Bax are members of the Bcl-2 family that play key roles in the regulation of apoptosis. The ratio between Bax and Bcl-2 often determines whether a cell will live or die. In this study, MCF-7 and T47D cells were treated with various concentrations of cisplatin for different times (24, 48 and 72h). Then, cell viability was assessed using MTT assay and IC50 was determined. RNA was extracted and cDNA was synthesized. Quantity of bcl-2 and bax genes expression compare to actb gene were analyzed using very sensitive quantitative Real-time PCR. bcl-2 gene expression was decreased and bax gene was increased in a dose- and time-dependent manner by cisplatin, that was statistically significant ($P<0.05$). The results showed that cisplatin exerted a dose- and time-dependent inhibitory effect on the viability, via up regulation of bax and down regulation of bcl-2 gene, in MCF-7 and T47D cells.

P06.052
Nurse-like cells support CLL lymphocytes survival in vitro via induction of SURVIVIN and BCL2 family genes.

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B-cell chronic lymphocytic leukemia (B-CLL), the most common leukemia in Europe and North America, is characterized by progressive accumulation of mature lymphocytes in peripheral blood, bone marrow and secondary lymphatic organs. This accumulation is due both to decreased apoptosis and increased proliferation. Altered mechanisms of programmed cell death and proliferation are not the intrinsic features of leukemic cells, but result from specific microenvironmental effect.
Histological markers of CLL are pseudofollicles (proliferation centers), dispersed in involved tissues. Pseudofollicles are made up from stromal cells, T lymphocytes, follicular dendritic cells, endothelial cells as well as others. These create protective microenvironment for CLL lymphocytes. Nurse-like cells (NLCs), first found in CLL mononuclear cells cultures, are also the part of this microenvironment.
Mechanisms that improve survival and increase proliferation of CLL cells are crucial in the context of effective therapy. The aim of the study was the analysis of proapoptotic genes expression on mRNA and protein level in CLL lymphocytes cultured with nurse-like cells.
PBMCs of 65 CLL patients were cultured for 14 days. Cell viability, apoptosis, proliferation, gene expression profile and selected proteins expression were assessed at day 0 and 14.
We found that NLCs support CLL lymphocytes survival in vitro, and the effect depends on NLC number. Gene expression profiling and RT-PCR showed the increase of proapoptotic genes *BCL2*, *BCL-XL* and *SURVIVIN* expression, what was further confirmed by their protein products analyses. However, no signs of CLL lymphocyte proliferation (any mitotic figures or Ki-67 expression) were observed.

P06.053
Genome-wide detection of copy number alterations in non-small cell lung cancer

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A great number of chromosomal aberrations detected in association with lung cancer allows to suppose that just a small part of lung cancer specific genes have been identified so far and there is still a lot to be examined and discovered in this field.
The aim of the present study was to develop a technique for genetic and morphological detection of somatic copy number variations (CNVs) in heterogeneous tumour tissue.
Whole-genome genotyping with HumanCytoSNP-12 BeadChips (Illumina Inc.) was applied to analyze non-small cell lung cancer (NSCLC) samples from 12 patients, which were then compared with 12 matching blood control samples screened for germline CNVs using Illumina HumanHap300 BeadChips. Four different algorithms (QuantiSNP, PennCNV, SOMATICs, BAFsegmentation) were used for

genotype analysis and the results were compared by applying Java programming language. CNV areas were plotted to the same graphics for visualisation of the results detected by four programs. That allowed choosing different analysing programs for different purposes and comparing in detail cancer tissue and germline copy number.
About 140 CNVs per patient were detected, most of which were amplifications. Some were found in more than one patient: 3q25-q27, 5p12-p14, 12p13, 9p13, 8p22. The results were further compared with gene expression data obtained from the same patients, and 30 genes with altered copy number showed expected changes in expression. A subset of the found CNVs was further confirmed by qPCR analysis.
The experiments demonstrated that these technique approaches for somatic CNVs detection in heterogeneous tumour tissue can be used for further researches.

P06.054
The *PABPC4L* gene, a candidate gene for colon cancer?

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In a large proportion of familial colon cancer cases, the underlying genetic cause for cancer predisposition remains unidentified. Recently, a 237 kb deletion on chromosome 4q28 was identified with array CGH in three individuals with microsatellite stable colon cancer from one family. Only one gene is present in this region: *PABPC4L*, a gene of which the function is unknown. To investigate the hypothesis that *PABPC4L* is a candidate gene for colon cancer, we investigated a group of patients with microsatellite stable colon cancer for germline mutations in *PABPC4L*. Ninety-three patients with colon cancer before the age of 50 were included in this study. Their tumours showed no microsatellite instability and normal mismatch repair protein stainings. In DNA isolated from lymphocytes, mutation scanning of the *PABPC4L* gene was performed with high-resolution melting analysis and direct sequencing. No pathogenic *PABPC4L* mutations were detected in the 93 patients. Therefore, our data do not support the hypothesis that *PABPC4L* is a candidate gene for colon cancer. Currently, analysis for large deletions is performed in 39 patients who did not have heterozygous polymorphisms.

P06.055
Analysis of different apoptosis ways by gene expression analysis and siRNAi silencing in colorectal cancer cells.

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Overexpression of apoptosis inhibitors is considered to be one of the main causative factor of apoptosis reduction and drug resistance of colorectal cancers. We set up a profile analysis of a number of genes related to apoptosis inhibition and cell proliferation in the colon cancer line HT-29: *c-IAP-2*, *cIAP-3*, *XIAP*, *Survivin*, *Livin*, *FLIP*, *HspA5*, *Gstp-1*, *Bcl2*, *c-myc*, *caspase 8*, *TRAP-1*, *Bcl2L*, *Kras*. The analysis has revealed at high level the expression of *TRAP-1*, *Survivin*, *XIAP* and *FLIP*. The oncogene *Kras* was found to be expressed even stronger compared with *TRAP-1* or *Survivin*. Most of tumors contain mutant allele *Kras* in the 2-nd exon. However no *Kras* mutation was found, arising a question of gene's hyperactivation in these cells. As far as cancer cell proliferation is dependent on *Kras* expression, we performed silencing by means of anti-*Kras*-siRNA. 38% of cell apoptosis was a result of this inhibition. The double decrease of cell viability (63%) was observed when used a combination of anti-*Kras, Survivin*, *FLIP* siRNAs. It is interesting that additive effect of three siRNA action was observed. This effect may serve for a good treatment strategy to induce apoptosis of colon tumor cells.

P06.056
Allelic imbalance of the *TGFβR1* is not a major contributor to the genetic predisposition to colorectal cancer

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An allelic expression imbalance of the *TGFβR1* gene was reported to be a strong risk factor for colorectal cancer (CRC) with an odds ratio (OR) at 8.7 (Valle *et al*, Science 2008) but several reports have reported contradictory results. We therefore conducted a prospective case-control study on carefully selected patients and controls. Patients were selected according to criteria suggestive of an increased genetic risk for CRC: (i) CRC before 61 years of age (or high-risk adenoma before 51) with a first degree relative presenting with CRC; *or* (ii) CRC before 51 years of age (or high-risk adenoma before 41); *or* (iii) multiple primitive colorectal tumors in the same case, the first one diagnosed before 61 years of age if cancer or before 51 if high-risk adenoma. The controls, aged from 45 to 60 years, were CRC-free and had no familial history of CRC. Allelic expression of *TGFβR1* was then measured, using SNaPshot and 4 SNPs (*rs334348*, *rs334349*, *rs1590* and *rs7871490*), by normalizing the ratio of the allelic peaks obtained on RT-PCR with the ratio of the peaks obtained on genomic DNA. Allelic expression ratios ranged between 0.82 and 1.41 in 98 informative controls and between 0.77 and 1.45 in 69 informative patients. No significant difference between patients and controls was observed. We concluded that allelic imbalance of the *TGFβR1* is not a major contributor to the genetic predisposition to CRC. Therefore, measurement of *TGFβR1* allelic imbalance has no clinical utility to identify patients with high CRC genetic risk.

P06.057
Verification of the Three Step Model in Assessing the Pathogenicity of Mismatch Repair Gene Variants

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To evaluate whether DNA mismatch repair (MMR) gene variations predispose to Lynch syndrome (LS), a three step assessment model has been proposed. Where LS is suspected, STEP1 is dedicated to the identification of the causative MMR gene and the variation. In STEP2, the functional effect of the variation is assessed in an in vitro MMR and in silico assays. Where LS cannot be confirmed or ruled out, more specific biochemical assays are required in STEP3. Here we verified the proposed model and its ability to distinguish pathogenic MMR variations from variants of uncertain significance by utilizing clinical, laboratory and in silico data of 37 MLH1, 26 MSH2 and 11 MSH6 variations. The proposed model was shown to be appropriate and proceed logically in assessing the pathogenicity of MMR variations. In fact, for MMR deficient MLH1 and MSH2 variations STEP3 is not required. STEP3 is important in assessing MMR proficient variations showing discrepant in silico results after STEP2. MSH6 variations require appropriate selection in terms of ruling out MLH1 and MSH2 variations and MLH1 promoter hypermethylation. Overall, taking into consideration the susceptibility gene, the three step model can be utilized in an efficient manner to determine the pathogenicity of MMR gene variations.

P06.058
SMAD7 rs4939827 risk variant is associated with poor outcome in colorectal cancer patients from R. Macedonia

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Several independent GWAS of colorectal cancer (CRC) have identified numerous disease loci that were not previously suspected to influence the risk of developing the disease. We performed a population based case-control association study on DNA samples from 350 CRC patients and 180 controls of 5 low-penetrant variants [rs10505477 (8q24.21), rs3802842 (1q23.1), rs4779584 (15q13.3), rs1800566 (16q22.1) and rs4939827 (18q21.1)]. Genotype-phenotype correlation of each variant with various clinico-pathological parameters (gender, age of onset, tumor location and TNM stage) was also analyzed. Only two SNPs, rs10505477 (8q24.21) (p=0,042, OR=1,372;

95%CI=1,010-1,862) and rs4939827 (18q21.1) (p=0.027; OR=1.831; 95% CI=1.066-3.144), confirmed population susceptibility to CRC in our population, whereas the other 3 showed trend towards, but did not reach statistically significant association. Phenotype/genotype correlation showed significant association of the 18q21.1 (rs4939827) T allele with age >60 (*p=1.662e-06;* OR = 2.665; 95% CI = 1.82-4.07) and advanced disease at diagnosis *(p*=0.0216; OR = 1.499; 95% CI = 1.058-2.092). RT/PCR analysis of paired blood/tumor samples showed that half of the patients had allelic imbalance at the 18q21.1 locus, with preferential selection of the risk allele in 2/3 of the patients. Our data add evidence that 8q24.21 and 18q21.1 genetic variants are markers predisposing to CRC in our population. We also demonstrate for the first time that enrichment of the risk T- allele of rs4939827 at 18q21.1 in tumors is associated with advanced disease at diagnosis, which further supports the notion that genes mapping in the vicinity to this locus are important regulators of colorectal cancerogenesis.

P06.059

Constitutional mono-allelic BUB1 deletion in a patient with non-classical mosaic variegated aneuploidy syndrome and early onset colorectal cancer

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Mosaic variegated aneuploidy (MVA) syndrome is a rare recessive disorder marked by growth retardation, microcephaly, childhood cancer and constitutive aneuploidy. Some cases of MVA have been associated with homozygous mutations in the BUB1B gene, a component of the spindle assembly checkpoint (SAC).

We have applied high-resolution SNP array profiling as an approach to identify novel cancer predisposing genes in patients with early onset mismatch repair proficient colorectal cancer. We encountered a constitutional mono-allelic deletion encompassing another important SAC gene, BUB1, in a patient who developed a colon carcinoma at age 37. Clinical examination revealed microcephaly and brachydactyly, two characteristic features of MVA syndrome. No mutations were found in the remaining BUB1 allele. In addition, no germline mutations in the APC gene were found. Since defects in the SAC are known to be associated with aneuploidy, metaphases of the patient's lymphocytes were analyzed and, by doing so, an increased level of constitutive aneuploidy (32%) was observed.

Regulation of the SAC has been shown to be dependent on gene dosage. Bub1 hypomorphic mice revealed reduced checkpoint activity and increased aneuploidy in a dose-dependent manner with increased spontaneous tumorigenesis. Furthermore, in a patient, who presented with early onset gastrointestinal cancer, a homozygous mutation in BUB1B leading to a reduced BUBR1 expression was recently reported. These data imply that, next to recessively inherited syndromes like MVA, haploinsufficiency of BUB1 or other SAC components may result in non-classical forms of MVA with a predisposition to develop gastrointestinal malignancies.

P06.060

Genetic profile of sporadic colorectal cancer liver metastasis vs. primary tumours by SNP-arrays.

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Most genetic studies in colorectal carcinomas (CRC) have focused on those abnormalities that are acquired by primary tumours, particularly in the transition from adenoma to carcinoma, while few studies have compared the genetic abnormalities of primary vs. paired metastatic samples. In this study we used high-density 500K SNP arrays to map the overall genetic changes present in liver metastases (n=20) from untreated CRC patients studied at diagnosis versus their paired primary tumours (n=20). Overall, metastatic tumours systematically contained those genetic abnormalities observed in the primary tumour sample from the same subject. However, liver metastases from most cases (n=8/20) showed acquisition of genetic aberrations that were not found in their paired primary tumors. These new metastatic aberrations mainly consisted of 1) an increased frequency of genetic lesions of chromosomes that have been associated with metastatic CRC (1p, 7p, 8q, 13q, 17p, 18q, 20q) and, more interestingly, 2) acquisition of new chromosomal abnormalities (e.g. losses of chromosomes 4 and 10q and gains of chromosomes 5p and 6p). These genetic changes acquired by metastatic tumours may be associated with either the metastatic process and/or adaption of metastatic cells to the liver microenvironment. Further studies in larger series of patients are necessary to dissect the specific role of each of the altered genes and chromosomal regions in the metastatic spread of CRC tumours.

P06.061

Effects of curcumin on global gene expression profile in the highly invasive human breast carcinoma cell line MDA-MB 231

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Introduction

Curcumin, or diferuloylmethane, is a major chemical component of turmeric (*Curcuma longa* Linn) that has been consumed as a dietary spice for ages. This yellow colored polyphenol has a surprisingly wide range of beneficial properties, including anti-inflammatory, antioxidant, antitumoral, anti-invasive and antimetastatic activity.

Materials and Methods

In this study, microarray gene expression analysis was applied to identify the curcumin-regulated genes in a highly invasive human breast-carcinoma cell line (MDA-MB-231). Cells were cultured (20μM) with curcumin for 24 hours; total RNA was isolated and hybridized to Agilent Whole Human Genome microarray slides. Gene Set Enrichment Analyses on our whole genome expression data revealed the downregulation of the EGF pathway elements followed by curcumin treatment.

Results

Furthermore, gene network analysis identified a significantly relevant network among the differentially expressed genes centered on the EGR-1 and FOS genes. The members of these pathway and network play an essential role in the regulation of cancer cell growth and development, most of them showed decreased expression level after the treatment of curcumin.

Conclusion

We have demonstrated the decreased expression of pathway elements of the EGF signaling cascade and the decreased expression of *EGR-1* gene on mRNA level in MDA-MB 231 human invasive breast carcinoma cell line. According to our knowledge we were the first who applied comprehensive GSEA and network analysis to analyze the gene expression profile of curcumin treatment on breast carcinoma cell line.These observations suggest that the use of curcumin may be an excellent candidate for prevention and treatment of breast cancer.

P06.062

High throughput validation of novel single nucleotide polymorphism associated with cytogenetically normal acute myeloid leukemia samples

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Acute myeloid leukemia (AML) is characterized by an arrest of maturation of hematopoietic progenitor cells. Patient prognosis varies widely and is currently stratified based on the presence or absence of specific cytogenetic abnormalities. Currently, cytogenetically normal AML is classified as intermediate risk, with a 5-year overall survival (OS) at 38. The overall 5-year survival rate is less than 50% in adults and significantly lower in the elderly (defined as > 65 years).

Here we describe a study with 4 cytogenetically normal AML patients - 2 of old age (defined as >65 years) and 2 young (< 45 years). Whole exome sequencing of these 4 samples was performed using Roche NimbleGen's SeqCap EZ exome capture and analyzed using Illumina's HiSeq platform. Novel discoveries were distinguished from constitutional single nucleotide polymorphisms (SNPs) by comparing each AML sample to match normal DNA from the same patient. Novel discoveries of the four patients, as well as SNPs that are

known to be associated with AML (i.e. FLT3, NPM1, IDH1, IDH2 and DNMT3A) served as input to a 13,500 custom SNP panel using Roche NimbleGen's AccuSNP custom genotyping platform. The custom SNP array serves as downstream validation of the whole exome sequencing discoveries and a tool to extent these findings to profile an additional 96 AML samples. This serves to demonstrate the power of a multi-tiered approach of combining targeting sequencing and flexibility of the AccuSNP platform to provide an accurate and high-throughput tool for identifying novel polymorphisms associated with AML.

P06.063
Analysis of frequency CCND1 gene polymorphism (rs3862792) in Polish patients with differentiated thyroid cancer

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The product of CCND1 gene belongs to the highly conserved cyclin family. Cyclins function as regulators of CDK kinases. Cyclin D1 forms a complex with CDK4 or CDK6, whose activity is required for cell cycle G1/S transition. This protein interact with tumor suppressor protein Rb and the expression of CCND1 gene is regulated positively by Rb. Mutations, amplification and overexpression of this gene, which influences on cell cycle progression, are observed frequently in a variety of tumors and may contribute to tumorigenesis.

Thyroid carcinomas are the most often carcinomas of endocrine system. Most often occurs papillary and follicular thyroid cancer: tumors with well prognosis, low benignity and slow progress but often giving recurrences and regional or remote metastasis and as well progression from well differentiated thyroid cancer to malignant anaplastic carcinoma.

We analyzed synonimic substitution c.669C>T (rs3862792) in CCND1 gene. Group of 628 patients (560 females and 68 males) with differentiated thyroid cancer and 378 individuals (185 females and 193 males) from population group were examined. Sequence variants were determined by pyrosequencing.

No significant differences in allele frequencies in patient with differentiated thyroid cancer and population group were observed. In DTC patient group allele T was present with frequency 0,024 and allele C with frequency 0,976. In population frequency for allele T was 0,037 and 0,963 for allele C. Project was supported by Polish Ministry of Science and Higher Education grant N N402 2874 36.

P06.064
Analysis of frequency ERCC2 gene polymorphism (rs3916891) in Polish patients with differentiated thyroid cancer

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Thyroid carcinomas are the most often carcinomas of endocrine system with still growing up frequency. Papillary and follicular thyroid cancer are the most frequent and belong to tumors with well prognosis, slow progress and low benignity but with tendency to recurrences and regional or remote metastasis.

In this focus, searching for molecular markers of disease course, good or poor prognosis and response on medical treatment is fundamental. It is expected that SNP polymorphisms research in genes demonstrating association with neoplastic diseases will be helpful in understanding of molecular mechanisms of thyroid gland tumors development and allow to better diagnosing.

We analyzed polymorphism c.*171G>C (rs3916891) in ERCC2 gene. Group of 455 patients (400 females and 55 males) with differentiated thyroid cancer and 320 individuals (144 females and 176 males) from population were examined. Sequence variants were determined by pyrosequencing.

We didn't observed significant differences in allele frequencies in patient with thyroid cancer and population group. In DTC patient group allele C was present with frequency 0,012 and allele G with frequency 0,988. In population frequency for allele C was 0,025 and for allele G 0,975

The differences were observed when considerate men and women separately. Allele C in males was observed with smaller frequency (0,011) comparing with females (0,042). In patient group we did not regard frequencies in these groups, because of much higher frequency of occuring differentiated thyroid cancer in woman. Project supported by Polish Ministry of Science and Higher Education grant N N402 2874 36.

P06.065
Analysis of frequency TNFa gene polymorphism (rs361525) in Polish patients with differentiated thyroid cancer

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TNFA gene encodes a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor superfamily. This cytokine is involved in the regulation of a wide spectrum of biological processes as cell proliferation, differentiation or apoptosis and has been implicated in a variety of diseases including cancer. The role of TNFalpha in cancer is complex with pro-tumourigenic and anti-tumourigenic role proposed. Differentiated thyroid carcinomas are the most often carcinomas of endocrine system with growing up frequency. In most cases occurs papillary and follicular thyroid cancer and more often it is present in women.

We analyzed polymorphism g.4752G>A (rs361525) in TNFA gene. Group of 607 patients 539 females and 68 males) with differentiated thyroid cancer and 482 individuals (282 females and 200 males) from population group were examined. Sequence variants were determined by pyrosequencing.

We didn't observed significant differences in allele frequencies in patient with differentiated thyroid cancer and population group. In DTC patient group allele A was present with frequency 0,055 and allele G with frequency 0,945. In population frequency for allele A was 0,04 and for allele G 0,96.

In this focus, very important seems to be searching for molecular markers of disease course, good or poor prognosis and response on medical treatment as well. It is expected that SNP polymorphisms research in genes demonstrating association with neoplastic diseases will be helpful in understanding of molecular mechanisms of tumors development and allow to better diagnosing. Project supported by Polish Ministry of Science and Higher Education grant N N402 2874 36.

P06.066
Dna damage in cultured human mesenchymal stem cells at various passages

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The cell therapy with multipotent mesenchymal stromal stem cells (MSCs) is promising treatment for various diseases. According to methodology the stem cells requires to cultivation before therapeutic use. It is known, spontaneous malignant transformation of MSCs in long-term culture is possible.

The DNA-damage can be plausible cause of chromosomal abnormalities and gene mutations. We studied the levels of DNA damage in MSC from human bone marrow at various passages of cultivation using single cell gel electrophoresis (comet assay).

Twenty eight samples of MSC's cultures from bone marrow of healthy donors have been analyzed. DNA damage was assessed as percent

of DNA in comet tail. The level of DNA damage and the frequency of apoptotic comets in cultures of MSCs studied at 3-4 passages and 10-11 passages.
The levels of DNA damage in MSC didn't change during culturing (3,5±0,9% on 3-4 passages and 4,4±1,2% on 10-11 passages). The frequencies of apoptotic comets in MSC cultures were 2,8±0,9% on the early passages and 3,6±1,8% on the late ones. In one of the cultures of MSCs we have observed a high level of DNA-damage (16,5% DNA in comet tail) and high rate of apoptotic comets (18,3%).

P06.067
DNA Microarray Analysis in Bulgarian Patients with Newly Diagnosed Glioblastoma Multiforme

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INTRODUCTION: Glioblastoma multiforme (GBM) is the most common and malignant primary brain tumor which is characterized by a number of different genetic alterations.
AIMS: We aimed to analyze the DNA profiles in an unselected group of Bulgarian patients with newly diagnosed GBM.
MATERIAL AND METHODS: Nine fresh-frozen tumor samples from patients with histologically confirmed primary GBM have been studied using a high-throughput DNA microarray analysis.
RESULTS: The most common numerical chromosomal aberrations were monosomy 10, 13, 14, 22, and trisomy 7 and 20. The most frequently detected copy number changes were deletions at 10p12, 10q23, 1p36, and 14q32; and gains at 7q36, 1q44, and 20q11-13. We identified a number of candidate tumor suppressor genes (KCNMA1, FGFR2, GFRA1, BNIP3, BACH, KLF12, VRK1, NF2, and OSM) and oncogenes (GNAI1, HGF, PCLO, SEMA3D, GRM3, RGS7, OPN3, CDK5, DNAJB6, ZNF658B, CDK5RAP1, and PTPN1), which are probably involved in the development of GBM.
CONCLUSIONS: GBMs are genetically heterogeneous group of tumors with unpredicted therapeutic response. The clinical significance of the DNA-microarray findings needs to be further established.

P06.068
Pharmacogenomics of cisplatin-based chemotherapy in ovarian cancer patients of different ethnic origin

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Background: Interethnic differences are becoming increasingly recognized as important factors accounting for interindividual variations in tolerability and efficacy of anticancer drugs. Although the reasons underlying ethnic diversity in drug responsiveness are likely multifactorial, inherited ethnic differences in drug-related genes may be, at least in part, the cause of the diversity.
Methods: 18 polymorphisms in 10 genes, the protein activities of which may be addressed in different aspects of cisplatin metabolism, were tested for correlations with efficacy and toxicity of cisplatin-based regimen (combination with cyclophosphamide) in Russian (n=104) and Yakut (n=87) women with epithelial ovarian cancer.
Results: There were significant ($p<0.05$) differences between Russian and Yakut patients in genotype distribution for GSTP1 Ile105Val, GSTP1 Ala114Val, *GSTA1* −69C>T, ERCC2 Asp312Asn, ERCC2 Lys751Gln, *ERCC1* 19007T>C, *CYP2E1* 96bp and *CYP2E1* −1053C>T. GSTP1 Ile105Val polymorphism was associated with progression-free survival ($p=0.004$) and overall survival ($p=0.02$) in Russian patients. In Yakuts correlation with progression-free survival were observed for *CYP2E1* 7632T>A polymorphism ($p=0.05$). The associations between genotypes and treatment toxicities were also different between ethnic groups. Russian patients with *GSTM1*-null and *GSTM3* intron 6 AGG/AGG genotypes had a lower risk of anemia. In contrast, in Yakut group anemia was less frequent among patients with *GSTA1* −69C/C genotype. Nephrotoxicity in Russians was associated with two polymorphisms in *ERCC1* gene but in Yakuts it was correlated with ERCC2 Lys751Gln polymorphism.
Conclusions: The marked ethnic differences in relevant pharmacogenetics may be a potential explanation for discordant platinum efficacy and toxicity in Asians and Caucasians, and are worthy of future studies.

P06.069
Polymorphisms of the DNA repair genes XRCC1 in acute leukemia

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In several DNA repair genes, polymorphisms may result in reduced repair capacity, which has been implicated as a risk factor for various types of cancer. The aim of the study was to investigate the association between genetic polymorphisms of XRCC1 and susceptibility to acute leukemia. We performed a case-control study involving 26 patients with acute leukemia (21 with myeloid leukemia and 5 with acute lymphoblastic leukemia) and 20 healthy controls for polymorphisms of codons 194 and 399 of the XRCC1 base excision repair pathway gene. Polymerase chain reaction (PCR) followed by enzymatic digestion of the PCR products was used for the genotyping of the two polymorphisms. The restricted products were analyzed on 2% agarose gel. The risk of development of acute leukemia was found to be significantly increased when variant XRCC1-399 (Arg/Gln) and XRCC1-194 (Arg/Trp) were present ($p<0.05$). These results suggest that the polymorphism of XRCC1 gene may contribute to the higher risk of acute leukemia.
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P06.070
Comparisons of MDR1 and MRP1 expression at RNA and protein levels using quantitative RT-PCR and Flow cytometry in breast cancer tumors

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One of the limitations in the treatment of cancer patients with chemotherapy is the development of multidrug resistance (MDR). This means that tumor cells become insensitive to a wide range of chemotoxic drugs which are structurally and functionally different. A well-known mechanism responsible for drug resistance is over-expression of ABC-transporter genes.The aim of the present study was to investigate the expression level of MDR1and MRP1 in breast cancer tumors and adjacent normal tissues of breast cancer patients at levels of RNA as well as protein levels and examine whether the expression of either or combination of these genes in breast cancer correlates with response to chemotherapy.
Fifty four patients with breast cancer who had undergone breast cancer surgery were enrolled in the study.Expression levels of MDR1 and MRP1in normal and tumoral breast tissues were evaluated by quantitative Real time PCR and flowcytometry.
Results showed that the expression levels of MDR1 and MRP1 in tumoral breast tissue were increased significantly than those of normal samples.
Our findings showed that evaluation of the expression at RNA or protein levels using Real time RT-PCR or flowcytometry are compareable but Real time RT-PCR is faster and cost benefit .

P06.071
A novel CDH1 transcript increases gastric cancer cell invasion and angiogenic potential

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Cell-cell adhesion plays a crucial role in epithelial tissue polarity and structural integrity and E-cadherin is a key player is this process. Reduced cell-cell adhesiveness results in destruction of the histological structure and increase invasion, the morphological hallmark of malignant tumors. Despite the strong correlation between E-cadherin impairment and malignancy, the cause underlying its impairment remains unknown in most epithelial cancers.In the present work, we identified a novel E-cad transcript, characterized its tissue specific expression and its functional role in a cancer context.A new E-cad exon was identified within intron 2 splicing with exon 3 at its canonical splice-site. 5'-RACE and CAGE analysis pinpointed two potential transcription start sites (TSS) and an ORF. This transcript includes all downstream canonical exons of E-cad gene, is expected to be translated and was named CDH1a. CDH1a is specifically expressed in spleen, a non-epithelial tissue and is absent from the stomach epithelia. In stomach cancer cell lines, it becomes overexpressed. Forced overexpression of CDH1a in cell lines expressing the canonical E-cad promotes cell invasion and angiogenesis and modifies their expression pattern as proved by microarray analysis. We identified a novel E-cadherin protein isoform cis-regulating the function of the canonical form. This finding may enclose a previously unrecognized mechanism leading to E-cadherin impairment in cancer.

P06.072

Frequency of EGFR mutations in Bulgarian patients with NSCLC

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Background: Lung cancer is the leading cause for death among many other cancer types. Non-small cell lung carcinoma (NSCLC) comprises approximately 80- 85% of all cases with lung cancer. According to publications in the last 5 years, some epidermal growth factor receptor (EGFR) mutations, correlate with positive response to treatment with tyrosine kinase inhibitors (TKI) such as genfitinib and erlotinib. While EGFR mutation profiles have been reported for certain Asian and Caucasian population there is no such report for Bulgarian population. It is established that 30- 40% of Asian and 10- 15% of Caucasian patients with NSCLC are carriers of EGFR positive mutations.

Aim: Establishing the frequency of EGFR mutations in Bulgarians with NSCLC.

Materials and methods: A group of 341 patients with NSCLC were analyzed so far. DNA was extracted from paraffin embedded tissues. We used high resolution melting (HRM) technology and subsequent sequencing of aberrant profiles to screen 73 patients for mutations in EGFR gene, the rest 268 patients were screened by qPCR based on Scorpions technology.

Results: We have detected TKI activating mutations only in 3% among the group of patients screened by HRM/Sequencing and in 11 % of patients, screened by qPCR. Deletions in 19th exon and two point mutations (L858R; L861T) in 21st exon were observed. Overall 9% of our patients were mutation positive.

Conclusion: Our study is in concordance with previous reports. However appropriate method for analysis has to be used.

P06.073

Complete transcriptomic, epigenomic and proteomic signature upon demethylation treatment of human breast cancer subtypes approached by multi-platform 'omics analyses

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Contribution of aberrant DNA methylation in tumorogenesis through silencing of tumor suppressor genes (TSGs) and microRNAs has been investigated. Since these epigenetic alterations can be reversible, it provides impetus to study both early and late effects of the 5-aza-2'-deoxycytidine (DAC) therapy in breast cancer using omics tools. Here we investigated multi-dimensional models to predict effects of DAC at the level of the genome, epigenome and proteome for subtypes of breast cancer.

The present study initially assessed the effective dosage of DAC for breast cancer therapy based on the cell viability, cytotoxicity, apoptosis and methylation quantification assays for different breast cancer cell lines. Then two breast cancer subtypes including highly aggressive and non-aggressive cell lines were investigated at various time points passages using multi-dimensional omics approaches (gene expression, microRNA expression and proteomics analysis). Complete molecular profile of the studied subtypes was identified. Additionally, the biological interactions and possible early and late systematic stable or transient effects of the methylation inhibition were determined in details.

The present study allows improving the effective optimal DAC dose cycles to establish intervals for epigenetic treatment of patients and also new DAC targets (including genes, miRNAs and proteins) and new involved pathways were identified. Furthermore, the results of the study give new therapeutic clues based on modification of pathological methylation patterns for management of cancer patients.

P06.074

New biomarkers detected for esophageal squamous cell carcinoma using chemiluminescent cDNA microarray gene expression profiling

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Esophageal cancer is the fifth leading cause of cancer related deaths in Iran. Esophageal squamous cell carcinoma (ESCC) is most often diagnosed in the advanced stages with a very low survival rates. No standard early diagnostic guideline has been proposed to date. Gene expression profiling using cDNA microarray can be an effective method in finding new molecular markers in ESCC. Ten samples including five tumors and five corresponding adjacent normal tissues of patients with ESCC were used for microarray analysis using Chemiluminescent Human Cancer GEArrays. Fifteen genes were detected with equal to or more than 1.5-folds significant overexpression compared to the normal tissue RNA. Thirty three genes showed equal to or more than 1.5-folds underexpression significantly (P<0.05). Overexpressed genes included AP2M1, CDC25B, FTL, HLA-C, HLA-G, HSPA8, ITGB4, MCM2, MMP2, PCNA and UBEL2L6 with a significant p-value (P < 0.05). Significantly underexpressed genes were CD24, RAP1A, RASGRF1, XRCC5 and TP53I3 (P < 0.05). AP2M1, FTL, UBE2L6, HLA-C and HSPA8 from overexpressed genes and XRCC5, TP53I3 and RAP1A from underexpressed genes have not been reported to date in gene expression profiling of ESCC. Validity Verification was performed using Real-time PCR for three randomly selected markers. MMP2 and HLA-G overexpression and XRCC5 underexpression were confirmed in 94 tumor and matched normal tissue samples of forty seven Iranian patients with ESCC, showing two tailed significancy of 0.008, 0.003 and 0.000 respectively. These molecular biomarkers may be suitable targets for further studies to find specific diagnostic, prognostic and therapeutic biomarkers especially in the Iranian population.

P06.075

Is there any association between risk factors obesity and occupational airborne exposures with p14 & p15 aberrant DNA methylation in esophageal cancer patients?

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It has shown that some risk factors for increasing of squamous cell carcinoma of esophagus such as obesity and occupational dust exposure could be affected on aberrant DNA methylation. For this reason, we studied for methylation at the p14 and p15 gene promoters by methylation-specific polymerase chain reaction assay (MSP) on DNAs extracted from 44 fresh tumor tissues and 19 non-tumor adjacent normal tissues, obtained from 44 patients affected by squamous cell carcinoma of esophagus (SCCE) in Iran. Statistical analysis was used to assess association of promoter methylation with bio-pathological, clinical and personal information data, including obesity and airborne exposures. The results showed that Methylation at the p14 and p15 gene promoters was detected in 9% and 41% of tumor samples respectively, but none of the non-tumor tissues exhibited the aberrant methylation. Moreover, we found the significantly associated with p14 promoter methylation and obesity (P=0.02) as well as p15 promoter methylation and occupational exposure (P=0.025). This study provides evidence that obesity and occupational exposure increase the risk of developing esophageal cancer through an enhancement of p14 and p15 promoter methylation.

P06.076

The functioning of important for tumor development genes in ovarian cancer tumor and its microenvironment.

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The estrogen receptor α (*ESRα*), osteopontin (*OSTP*) and *BRCA1*, *BRCA2* gene expression in samples of ovarian cancer, in tumour microenvironment and in distant from tumour regions were investigated. In accordance with *ESRα* and *OSTP* role in cancer development, their expression in tumor relatively normal ovarian tissue was increased in 35-40% of cases. Important, that in tumour microenvironment these genes were activated higher than in tumor in 25-30% of cases, sometimes even if the expression in tumor was near to normal. Possibly this reflects the postulated effect of tumor instigation by microenvironment and should be taken into account in practical diagnostics, especially ESR. The *BRCA1* role in *ESRα* and *OSTP* regulation as it was observed for breast cancer was not found for ovarian cancer in our work. However we revealed a correlation of *BRCA2* gene expression with both *ESRα* and *OSTP* expression in ovarian cancer tumor. In this aspect it is interesting inverse correlation of relations of expression levels in tumor and microenvironment for *BRCA1* and *BRCA2* revealed by us. The data for the first time highlight the functional interrelation of important for tumor development genes in the ovarian tumor and its microenvironment.

P06.077

Methylation of estrogen receptor alpha (ERα) gene promoter is a frequent event in Iranian women with sporadic breast cancer

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Breast cancer affects Iranian women at least one decade earlier than western patients with noticeably different clinical manifestation. Breast tumors are classified based on estrogen receptor alpha status to ER negative (30-40%) and ER positive. Considering to the challenging nature of ERα- tumors treatment, and its innate poor prognosis, clarification of the molecular mechanisms that control expression of ERα are essential. Aberrant methylation of gene promoter region is one of the mechanisms for gene silencing in breast tumors which are potentially reversible and so they are good targets for new therapeutic strategies. For analyzing the methylation status of ERα gene, ER3 region were assessed with methylation specific polymerase chain reaction (MSP). ERα methylation was detected in 64 of 91(70%) breast tumors. A strong correlation was found between ERα methylation in ER3 region and ER negativity in tumors as 88.9% of ER negative versus 52.2% of ER positive cases were methylated (P< 0.00001). There were significant correlations between methylation in ER3 region and negative status of progesterone receptor in tumors (P< 0.01). Presence of ERα methylation in a sizable fraction of ER+ cases, maybe due to cellular heterogeneity in breast tumors. ER positivity in breast tumors is a dynamic phenotype and over the natural course of cancer progression, ER can be lost and many ER+ tumors become ER- in the course of the disease. Presence of ERα methylation in ER+ tumors is a manifestation of this heterogeneity and may contribute to endocrine therapy resistance or recurrence.

P06.078

Molecular sub typing of breast tumors in Iranian breast cancer patients and its association with estrogen alpha receptor (ERα) methylation

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Breast cancer is one of the most frequent malignancies among Iranian women and its age of onset seems to be about 10 years earlier in comparison to European countries. This cancer is a heterogeneous disease that includes many morphological and molecular entities. We had shown that ERα promoter methylation is a frequent event in Iranian patients. Therefore we investigated the association of this epigenetic event in different molecular subtypes of breast tumors. Although breast cancer subtypes were originally identified by gene expression analysis using DNA microarrays, large-scale sub typing using gene expression profiling from formalin fixed, paraffin-embedded samples is not currently feasible. According to recent approaches about immunohistochemical (IHC) based classification of breast tumors to molecular subtypes, we used IHC surrogates to identify breast tumor intrinsic subtypes in 91 breast tumors in Iranian patients. ER-/PR-/Her2- tumors were classified as triple negative (basal like) (25%), ER-/PR-/her2+ tumors as her2+ group(24%), ER+/PR+/Her2- as luminal A (34%) and ER+/PR+/Her2+ as luminal B tumors(17%). Methylation status of estrogen receptor promoter was investigated in these subtypes. There was a significant correlation between methylation in ERα and triple negative subtype where 20/23 (86.7%) of them were methylated. Also significant correlation were found in Her2+ subtype tumors with 20/22 (90.9%) methylation in ERα (*P*<0.002). These two non-luminal subtypes have poor prognosis and it has shown that they are associated with significant decrease in survival.

P06.079

APC germinal mosaicism in a patient with Familial Adenomatous Polyposis carrying the c.4666del mutation

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We report a case of germinal APC mosaicism in 36 years-old male patient diagnosed for Gardner syndrome with features of adenomatous polyposis and without family history of polyposis. No mutation in APC gene was initially found. The APC gene of his daughter was sequenced because of the occurence of osteomas at five and nine years old, and the germline heterozygous mutation c.4666del was detected. This mutation was detectable in father's lymphocytes DNA but in low proportion (9%), suggesting the presence of somatic mosaicism in the father. The detection of the mutation in other tissues (buccal swab, normal colon tissue) and in the offspring suggests that it may have occurred early during embryogenesis, before the separation of the embryonic layers. As expected, the higher level of mutated allele was detected in adenomas. Several dilutions of mutated DNA in wild

type DNA show that the High Fusion Resolution (HRM) technique allows a 2.5% proportion of the mutated allele to be detected. Sanger sequencing alone is not sensitive enough to detect low mosaicism levels and pre-screening methods like HRM could be used for APC analysis. Mosaicism is an important consequence of the high rate of *de novo* APC mutations and must be considered in the diagnosis of apparently *de novo* cases. Mutation screening of offspring with the disease could be preferred when possible because of the risk of mosaicism. When no mutation is detected it can be envisioned to sequence adenomas DNA as a first step toward identifying APC mosaicism.

P06.080
Mutations of APC and MYH Genes in patients with Familial Adenomatous Polyposis in populations from Central Western Spain and Canary Islands

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Introduction: Germline mutations in adenomatous polyposis coli (APC) are associated with the development of familial adenomatous polyposis (FAP), a hereditary colorectal cancer syndrome with autosomal dominant inheritance pattern. This syndrome is characterized by the presence of hundreds to thousands of adenomatous polyps in the colon and rectum. Attenuated familial adenomatous polyposis (AFAP), is related to mutations in the end 5 ‘, in exon 9 and in the end 3’ of the APC gene. Biallelic mutations in MYH gene have also been identified in patients with multiple colorectal adenomas and in APC-negative patients with FAP.

Materials and Methods: We collected samples from peripheral blood to probands belonging to 55 families of high risk for familial adenomatous polyposis. Genomic DNA was extracted from peripheral blood cells. Amplified PCR products were analyzed by CSGE, dHPLC, restriction enzymes, MLPA, In silico analysis and direct sequencing.

Results: Molecular analysis of APC gene, has allowed the identification of pathogenic germline mutations in 75% of FAP families and 17% of AFAP families. We found 41 different mutations in the APC gene, which have been cataloged in pathogenic mutations, variants of unknown significance and neutral polymorphisms. In MYH gene, the analysis revealed 6 different pathogenic germline mutations in 7 unrelated families, including 6 families with biallelic and 1 with monoallelic variants.

Conclusions: We described 14 pathogenic mutations in APC gene; eight of them are reported for the first time. Additionally, we confirm that germline mutations in MYH gene are responsible for a significant fraction of colorectal polyposis.

P06.081
Mutation screening of *PALB2* gene in breast/ovarian cancer families by Enhanced Mismatch Mutation Analysis (EMMA).

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A family history of breast cancer is one prerequisite criterion for testing for *BRCA1* and *BRCA2* germ-line mutations. However, germ-line mutations in *BRCA1-BRCA2* genes account for only 15-20% of breast cancer families. Recently, the *PALB 2* gene, known as one of the Fanconi anemia genes (FANCN), has been identified as a Partner and Localizer of *BRCA2* and was reported to contribute to breast and pancreatic cancer susceptibility. Monoallelic germ-line mutations in *PALB2* have been reported in 1% to 3% of early-onset and/or familial cancer cases and confer more than a two-fold increased risk to develop breast cancer.

In a series of patients previously analysed for *BRCA1/BRCA2* with no detectable point mutation or large rearrangement, we screened 94 index patients for germ-line mutations in *PALB2* by EMMA (Enhanced Mismatch Mutation Analysis). EMMA is, a new fast and cost-effective method based on heteroduplex analysis by capillary electrophoresis. The 13 coding exons and intron-exon junctions of *PALB2* were amplified as 19 amplicons in 6 multiplex PCR using a single condition. To validate the EMMA method we sequenced directly the exons for all samples. We did not observe any discrepancy between the two methods. We identified one truncating *PALB2* mutation in a family with three breast cancer cases (mean age 53 years) and 1 ovarian cancer case. Four novel missense mutations were also identified.

This study is the first report of *PALB2* mutation screening on French breast-ovarian cancer families. Although *PALB2* mutations seem rare, their contribution to hereditary breast/ovarian cancer susceptibility needs further investigation.

P06.082
Familial Esophageal Squamous Cell Carcinoma and Breast Cancer in Several Pedigrees with BRCA2 Mutations

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Esophageal squamous cell carcinoma (ESCC) is the 6th most common fatal cancer in men and 9th in women in the world And the 5th leading cause of cancer in Iran. A high frequency of ESCC in northeastern Iran has been reported. BRCA2 may play an important role in ESCC carcinogenesis as indicated study from Iran, India and China suggested that. The incidence of multiple primary cancers is reported between 0.3% and 4.3%. Sequential primary ESCC followed by primary Breast Cancer (BrCa) has not been reported previously. Mutational analysis of BRCA2 in several familial ESCC/BrCa pedigrees with affected ESCC individuals who later contracted BrCa was performed.

Individuals with ESCC were treated successfully by surgery and chemotherapy, however, after several years some of the pedigree members contracted invasive ductal carcinoma BrCa. We screened patients for BRCA2 mutations in germline DNA by whole gene sequencing. Some known BRCA2 mutations and a novel splice variant, c.426-2A>G were detected. Novel mutation tracking ruled out SNP in 50 healthy individuals. The related family members, 60 sporadic and 12 familial ESCC were negative for this new mutation. The novel splice variant mutation was found in two affected ESCC/BrCa sisters, 54 yrs and 48 yrs old, and their sons and daughters, two brothers and two nephews. Sequencing of BRCA2 cDNA, exons 4 to 7, revealed that c.426-2A>G mutation lead to exon 5 skipping in splicing process.

These results intrigued a possible link between ESCC and BrCa through BRCA2 tumorigenesis pathway.

P06.083
Identification of a homozygous nonsense mutation of FANCM in a case with polycythemia vera carrying uniparental disomy 14q

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Polycythemia vera is a chronic myeloid neoplasm characterized by elevated erythrocyte mass and susceptibility to acute myeloid leukemia (AML). Acquired uniparental disomies (UPD) are frequent lesions in cancers that were shown to amplify heterozygous somatic mutations. UPDs of the long arm of chromosome 14 (14qUPD) are frequent defects in myeloid neoplasms, however, the mutant gene target remains unknown. In an attempt to find the target of 14qUPD we performed exome sequencing on tumor DNA sample from a patient with polycythemia vera and carrying 14qUPD. We analyzed the exome sequencing data for point mutations and focused on the chromosomal region of 14qUPD. A homozygous nonsense mutation, R658X, was found in gene *FANCM*. Germline mutations in *FANCM* cause Fanconi anemia and an increased susceptibility to AML. We confirmed the

homozygous mutation in myeloid cells by Sanger sequencing, while the buccal mucosa control sample was heterozygous for the mutation. Clinical data showed that the patient was initially diagnosed with polycythemia vera and had high hemoglobin levels, while at sample the patient was anemic and later transformed to AML. It is unclear whether the nonsense mutation in *FANCM* caused proliferative advantage in the patient or there is a second, somatic mutation that drives the clonal outgrowth. In any case, acquired homozygosity for the germline mutation changed the clinical course of the disease. The interplay of somatic genetic events and germline deleterious variants provides another dimension in clonal evolution of cancers.

P06.084
Novel mutation and polymorphism of the APC gene in patients with FAP

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Background: Colorectal cancer which has become specially prevalent in developed countries. Familial adenomatous polyposis (FAP) is an autosomal dominant disease characterized by hundreds of adenomatous polyps in the colon and rectum and by a variety of extracolonic features.Mutation of the adenomatous polyposis coli (APC) gene, a tumor suppressor , is though to be an early event in patients with FAP.
Method: This study was conducted on 15 patient with FAP. Genomic DNA was extracted according to standard salting out protocol. Whole coding region of the gene was scanned using bidirectional sequencing analysis.
Results: In the present study the screening of APC gene led to the identification of two novel missense mutations and one novel polymorphism. The mutations were including a A>G transition (AAG→AGG) at nucleotide 6226 in exon 15. This alteration substitute an arginine to the normal lysine at residue 2057 (Lys 2057 Arg). The other mutation, G>C transition (AGC→ ACC), was identified at nucleotide 6772 in exon 15. This alteration replaces serine with threonine at residue 2239 (Ser 2239 Thr) in another proband. Moreover, 1novel polymorphism determined in codon 15, C>G transition (ACT→ AGT) at nucleotide 6820. It causes replacement of threonine with serine at residue 2255 (Thr 2255 Ser).
Conclusions: Our findings showed that probably the mutations have been located in these regions are responsible for some cases of FAP deficiency. Therefore, we could consider these types of mutations in order to predict individuals at risk of FAP in our population.

P06.085
The APC gene mutation analysis in Familial Adenomatous Polyposis, a large cohort study from Iran

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Familial Adenomatous Polyposis (FAP) is a rare dominant disease accounting for approximately 1% of all colorectal cancers. The gene responsible is APC mapped to chromosome 5q21-22 and isolated by positional cloning. APC is a tumour suppressor gene which is inactivated as a gate keeper in either familial or sporadic FAP. In this study the germline mutations in FAP patients determine possible genotype-phenotype correlations in these cases. The germline mutations were detected in 63% of patients using heteroduplex analysis on genomic DNA and cDNA. This is the first report of molecular analysis of FAP in a large cohort study from Iran.

P06.086
Prognostic and predictive significance of the bcl -2/IgH translocation in malignant follicular lymphomas

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In Europe, follicular lymphomas constitute up to 30% of non-Hodgkin lymphomas.
Prognostic markers, identified by 2 different working groups, immunohistochemical (CD10, bcl 2 positivity) and molecular (the bcl 2/ IgH hybrid gene, the t(14;18)(q32:q21.
The aim of our study was to analized the cytogenetical aberrations in malignant follicular lymphomas, in order to identify the prognostic and predictive value of bcl2-2/IgH translocation in these malignancies.
Material and method: We conducted a study on 79 patients with follicular lymphomas. The study was carried out on tissue samples selected from the “Victor Babes” National Institute of Pathology files. These samples were formalin-fixed paraffin embedded-tissues, routinely processed for histology.
The t(14;18) translocation, realized by the bcl 2/IgH rearrangement, supposedly occurs in almost all follicular lymphomas (FL) can be detected by FISH methods
We employed the PathVysion LSI IGH Spectrum Green/ LSI bcl2 Spectrum Orange (VYSIS) kit.
Results: Asingnificant positive correlation was found between the IHC positivity for bcl 2 and the FISH detection of t (14;18) translocation (p=0,04).
Twenty two cases were selected for FISH analysis, five cases were excluded because of the processing artifacts , ten of the 17 cases without artifacts (58.8 %) presented t(14:18) translocation: two cases of FL grade 1-2, two cases of FL grade 1, six cases of FL grade 3.
In 66.6% of cases with t(14;18) translocation, the immunohistochemical reaction for bcl 2 protein was positive.
Bcl2 t(14;18) translocation plays an important role in the pathogenesis of follicular lymphoma, and is an important tool in the diagnosis and treatment.

P06.087
FLT3 internal tandem duplication and *FLT3-D835* mutation in 80 AML patients categorized into cytogenetic risk groups.

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Acute myeloid leukemia (AML) is a clonal disorder characterized by various genetic abnormalities and variable response to treatment. About 50% of patients with AML have no cytogenetic aberrations, presenting normal karyotype, and are categorized in the intermediate risk group. In this group detection of FLT3 mutations move a patient from the intermediate to the adverse risk group. Bone marrow from 80 AML patients was cultured to obtain chromosome slides and then karyotype. Simultaneously DNA was isolated from bone marrow and PCR reaction was conducted to test the FLT3 mutation status (ITD and D835). For statistical analysis Chi squared test was used. From the group of 80 AML patients seven were classified as a favorable risk group and FLT3/ITD was found only in one of these patients (14.28%), and FLT3/D835 in another one (14.28%). Fifteen patients showed a complex karyotype with more than three aberrations or with any aberration known as a poor prognosis. Among the adverse group FLT3/ITD was detected in three patients (20%) and D835 mutation in two other patients (13.33%). Among 58 patients with normal karyotype in GTG banding FLT3/ITD occurred in six cases (10.34%) and D835 mutation in two cases (3.45%). No significant difference was found among these three risk groups regarding presence or absence of FLT3/ITD and FLT3/D835. Molecular characterization of mutations in several genes, such as FLT3, NPM1, MLL, CEBPA, in acute myeloid leukemia, especially in normal karyotype cases, could be another factor after cytogenetic analysis to stratify AML patients into different prognostic categories.

P06.088
FOXE1 locus is a major genetic determinant for radiation-induced papillary thyroid carcinoma

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After the Chernobyl nuclear power plant accident in April 1986 an increase in thyroid cancer incidence, essentially papillary thyroid carcinoma (PTC) in children, occurred in the contaminated areas. Previous studies demonstrate that children exposed to ionising radiation show an increased risk of PTC, and carry it with them into adult life. Given the inter-individual variation in response to radiation dose and familial aggregation of thyroid cancer, it is likely that genetic factors affect radiation-related thyroid cancer.
In 1999, the RAD group at IARC set up a population-based case-control study in Belarus and the Russian Federation to evaluate the risk of thyroid cancer after radiation exposure in childhood. The study aimed to investigate how environmental and host factors interplay to modify this risk. In the current analyses, we included 413 subjects from Belarus (83 PTC cases and 330 matched controls); all of them were aged 15 or younger at the time of the accident. We genotyped, in this unique series, 8 SNPs shown to be associated with sporadic PTC: rs965513 (nearby *FOXE1* on 9q22.33), rs944289 (*NKX2* on 14q13.3), rs4704397 (*PD8B*), rs2252696 (*TG*), rs2910164 (Pre-miR-146a), rs2145418 (1q12), rs4658973 (*WDR3*), rs4903957 (*TSHR*).
Consistent with findings from the Genome Wide Association Studies (GWAS), the SNP on 9q22.33 appears to double the risk of radio-induced PTC for carriers (OR=2.39 [1.22-4.68], p=0.026). By contrast, the second GWAS SNP on 14q13.3 and the other candidate SNPs were not replicated in this series.
Further studies are underway to understand the role of *FOXE1* in thyroid carcinogenesis.

P06.089
A novel pathogenomic mechanism in FPD/AML by a constitutional t(16;21) involving 16p13 ATF7IP2 and 21q22 RUNX1 loci

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Familial platelet disorder with propensity to myeloid malignancy (FPD/AML) is an autosomal dominant disorder characterized by platelet abnormalities and a predisposition to acute myeloid leukemia (AML). FPD/AML is caused by constitutional point mutations and copy number changes in 21q22 *RUNX1*. Given the clinical heterogeneity of FPD/AML, the syndrome could be overlooked. In the past this has resulted in stem cell transplantation (SCT) of patients from affected siblings before detection of the inherited *RUNX1* mutation. Therefore, adequate diagnostics are essential to exclude FPD/AML. The identification of novel mutations is required to define clinical features and could contribute to risk stratification given the heterogeneous manifestation of this rare disorder. Here we report novel p.Gly143Arg and p.Gln235X (*RUNX1b* variant) mutations in two pedigrees. In a third pedigree no *RUNX1* anomaly was detected by sequencing or copy number analyses using array CGH. We hypothesized that FISH analysis could reveal a balanced 21q22 *RUNX1* translocation. A rearrangement of the *RUNX1* locus by a constitutional t(16;21)(p13;q22) was identified as a novel pathogenomic mechanism in FPD/AML. The 16p13 breakpoint was confined to the proximity of the *ATF7IP2 (MCAF1)* locus, encoding a transcription factor involved in proliferation in human cancer by epigenetic transcriptional control of Sp1-dependent maintenance of telomerase activity. This is the first report on a constitutional chromosome translocation with an oncogene rearrangement as a pathogenomic mechanism in a hematological malignancy. Our data indicate that FISH analysis of *RUNX1* for structural aberrations has to be included in the FPD/AML diagnostic sequel.

P06.090**
MicroRNA-101 modulates E-cadherin expression and function through EZH2 up-regulation in gastric cancer

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Inactivation of E-cadherin (*CDH1*), by genetic and epigenetic mechanisms, is recognised as a pivotal step in gastric cancer initiation and progression. Yet, a considerable proportion of gastric cancers lack classical *CDH1* inactivation mechanisms (*CDH1* mutation, loss of heterozygosity and/or DNA methylation), but nevertheless loss or mislocalisation of E-cadherin expression is still frequently observed. To address this issue, we explored an indirect pathway involving chromosomal abnormalities, a microRNA (miR-101) and a histone methyltransferase (EZH2), which may contribute to E-cadherin impairment in sporadic gastric cancer. We confirmed that, independently of the histological type, E-cadherin is frequently impaired (95%) in our series of sporadic gastric cancer. However, only 30% of the cases harbour classical inactivation mechanisms. We found that miR-101 expression is downregulated in most of these cases and proved, for the first time, that this occurs via (micro) deletions at one of the microRNA-101 loci in chromosome 9p24.1 (miR-101-2 locus). Moreover, 40% of cases showing decreased levels of miR-101 displayed overexpression of EZH2 (a known target gene of miR-101) which, in turn, associates with loss or aberrant E-cadherin expression. Conversely, we demonstrated that depletion of EZH2, by short interference RNA, rescues E-cadherin cell-membrane expression. Overall, we show that loss of miR-101 with parallel EZH2 upregulation may constitute an additional mechanism that can either directly impair E-cadherin expression or may co-exist with classical E-cadherin inactivating mechanisms seen mainly in advanced cancers with intestinal morphology.

P06.091
The IL1-RN +2018T>C polymorphism is associated with increased susceptibility to gastric adenocarcinoma

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Gastric cancer is the fourth most common cancer and the second most common cause of cancer deaths worldwide. Gastric carcinogenesis is a complex, multistep process, which may be influenced by many factors, including genetic variations. The aim of our study is to investigate the IL-1B -31T>C (rs1143627), IL-1B +3954C>T (rs1143634) and IL1-RN +2018T>C (rs419598) polymorphisms and gastric cancer susceptibility in Eastern Europe (Romanian population), a region in which the association between gastric cancer and these polymorphisms has not previously been studied. A total of 103 patients with gastric adenocarcinoma and 244 healthy controls were included. DNA was extracted from the blood specimen. These polymorphisms were genotyped by allelic discrimination TaqMan PCR. Allelic distributions were examined for deviation from their corresponding Hardy-Weinberg equilibrium. The effects of cytokine alleles on the risk of diseases were expressed as odds ratios (OR) with 95% confidence intervals (CI). A significant association was observed for IL-1RN+2018T>C, the subjects carrying CC genotype were at a 2.5 fold elevated risk for gastric cancer (OR 2.52, 95%CI:1.06-6.01). Separate comparisons between tumor site and controls, and between histology type cancer and controls also showed that IL1-RN+2018T>C was associated with an increased risk of gastric non-cardia adenocarcinoma (OR 2.77, 95%CI:1.10-6.99) and intestinal type (OR 3.08, 95%CI:1.17-8.11). No significant difference was observed between all gastric cancer cases and controls or in stratified analysis for both IL-1B polymorphisms. In conclusion, polymorphism IL1-RN+2018T>C may contribute to gastric cancer risk, mainly for non-cardia and intestinal types of gastric adenocarcinoma in Eastern Europe.

P06.092
Mutations in gastrointestinal stromal tumors in the Slovak population - a population based study.

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Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the gastrointestinal tract, characterized by

expression of CD117 and harboring activating mutations in *KIT* and *PDGFRA* protooncogenes. In this study, we analyzed the distribution of 192 primary mutations identified in 192 patients with GIST in the Slovak population from April 2007 till June 2010. The exons 9, 11, 13 and 17 of *KIT* and exons 12, 14 and 18 of *PDGFRA* were examined using direct dideoxysequencing after PCR amplification. From these 192 mutations, the *KIT* mutations were present in 160 cases (83%) and *PDGFRA* in 32 cases (17%). The most common were exon 11 mutations in 137/160 cases with 84/137 deletions, 37/137 point mutations and 16/137 duplications/insertions. The deletions within exon 11 were divided into two groups, small deletions (<20 bp) and large deletions (>20 bp) and the most affected codons were 558 and 570, respectively. Subsequently, twenty mutations were identified in exon 9, the most common p.A502_Y503dup in 18 cases. Within exons 13 and 17, we found two p.K4642E missense mutations and one p.N822K substitution, respectively. The most common mutation in *PDGFRA* was the p.V842D (18/32) in exon 18. The distribution of mutations in patients with GIST in Slovak population is similar to the previous reports. Analysis of these mutations is important for indication of the targeted therapy and is emerged as a paradigm for genotype-driven therapy.

P06.093

Impact of *SHMT* C1420T polyorphism in head and neck cancer

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INTRODUCTION: Head and neck squamous cell carcinoma (HNSCC) is considered one of the most frequent tumors in developing countries and the main risk factors are tobacco and alcohol. Alterations in folate metabolism may contribute to the process of carcinogenesis by influencing DNA methylation and genomic stability. Polymorphisms in genes encoding enzymes involved in this pathway may alter enzyme activity and consequently interfere in concentrations of homocysteine, S-adenosylmethionine and other metabolism products that are important for DNA synthesis and cellular methylation reactions. The objective was to investigate *SHMT* C1420T polymorphism involved in folate metabolism on head and neck cancer risk and the association between this polymorphism with risk factors. **PATIENTS AND METHODS:** Polymorphism was investigated in 731 individuals (241 patients and 490 controls) by Real Time-PCR. Chi-square and Multiple logistic regression were used for the statistical analysis. **RESULTS:** Multiple logistic regression showed that tobacco, male gender and age over 48 years were predictors for the disease (P<0.05). *SHMT* C1420T polymorphism associated to risk factors were not related to HNSCC (Table 1). **CONCLUSION:** The present study suggests that tobacco, male gender and age over 48 years modulate HNSCC risk. However, further investigation of gene interactions in folate metabolism and studies in different populations are needed to investigate HNSCC risk.

Table1. Distribution in OR of gender, age, polymorphism and risk factors between HNSCC patients.

Variables	Patients N (%)	Controls N (%)	OR (95%CI)	P value
Tobacco consumption				
Non-smokers	41 (17.02)	295 (60.20)	Reference	Reference
Smokers	200 (82.98)	195 (39.80)	3.82 (2.45-5.97)	**P<0.05**
Alcohol consumption				
Alcohol non-consumers	67 (27.80)	245 (50)	Reference	Reference
Alcohol consumers	174 (72.20)	245 (50)	1.41 (0.92-2.18)	P=0.11
Gender				
Female	29 (12.03)	142 (28.98)	Reference	Reference
Male	212 (87.97)	348 (71.02)	1.72 (1.02-2.92)	**P<0.05**
Age				
< 48 years	36 (14.94)	330 (67.35)	Reference	Reference
> 48 years	205 (85.06)	160 (32.65)	8.60 (5.64-13.10)	**P<0.05**
***SHMT* C1420T genotypes**				
CC	124 (51.45)	260 (53.06)	Reference	Reference
CT	98 (40.66)	183 (37.35)	0.87 (0.59-1.27)	P=0.46
TT	19 (7.89)	45 (9.59)		

P06.094

Genistein-induced miR-23b expression via hyperthermia inhibits the growth of breast cancer cells

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MicroRNAs (miRNA), non-coding RNA molecules, play a major role in the regulation of gene expression. Mature miRNAs cause mRNA degradation or inhibition of protein translation via complementary or partly binding to the mRNAs, so they act as oncogenes and tumor suppressor genes.

Genistein, an isoflavonoid, is a prime anti-cancer component of soybean and plays roles in the inhibition of protein tyrosine kinase phosphorylation, tumor progression, apoptosis, induction of cell differentiation and disruption of free radicals. Hyperthermia is the exposure of cells to higher temperatures (41-44°C) than normal psychological degrees.

This study aimed to induce oxidative stress by exposing MCF-7 breast cancer cell line to hyperthermia and determine the IC_{50} dose of genistein and evaluate the combined effect of this isoflavone and hyperthermia in terms of miRNA expression levels.

The IC_{50} dose of genistein was 175μM and hyperthermia caused 40% cytotoxicity in MCF-7 cell line. The combination of hyperthermia and genistein showed highest cytotoxicity during the 72nd hour of the experiment.

miRNA expression profilings of 88 miRNAs and 4 housekeeping miRNAs were quantified using real-time online RT-PCR. miR-23b, a miRNA which is reported to be down-regulated in human cancers, was found to be up-regulated 56.69, 739.29 and 918.10 folds following the treatment of genistein, hyperthermia and genistein-hyperthermia combination respectively, compared to the control group of genistein-free cells not exposed to hyperthermia.

In conclusion, up-regulation of miR-23b may offer an alternative miRNA-based therapeutic strategy, as an additional protocol for the treatment of breast cancer.

P06.095

Investigation of the Inhibitory Effect of Soy-derived Genistein on the Metastasis Potential of A549 Lung Cancer Cell Line

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The lung cancer is one of the most dangerous cancer in the world . There are two main types of lung cancer: non-small cell lung cancer and small cell lung cancer.

The metastasis of lung cancer is highly dependent of expression of matrix metalloproteinases, and correlated with phosphorylation of ERK1/2 and PI3K/Akt pathways. Here, we showed that genistein decreased the migration and invasion of human non-small cell lung cancer cells (A549 cell line) in vitro, because the combination of anti-cancer drugs with nutritional factors is a potential strategy for altering to increase the efficacy and decreasing the toxicity of chemotherapy. This study examined the effects of genistein on the level expression of mRNA and protein of MMP-2 in lung cancer A549 cell line by qRT-PCR and Zymography.The cytotoxicity effects and proliferation rates does-dependent of genistein in this cell line were determined by MTT and LDH assays. A549 cells were treated for 24, 48 and 72

hrs, with genistein concentrations of 0, 25, 50, 75 and 100 mmol/ml. These results have shown that the most effect of inhibition in growth and proliferation of A549 cell line is 75 mmol/ml concentration. Also, these results indicate that genistein downregulates MMP-2 mRNA and protein expression in A549 cell line, and result of inhibition of ERK1/2 and PI3K/Akt pathways phosphorylation by ELISA indicated that genistein inhibited phosphorylation rate of both pathways. Therefore that it can decrease recurrence and metastatic rates in lung cancer. This issue should be further examined for the clinical treatment.

P06.096**

Germline epigenetic silencing of the tumor suppressor gene *PTPRJ* in early onset familial colorectal cancer

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INTRODUCTION: Recently we showed that 3' deletion of the *EPCAM* gene leads to allele-specific epigenetic silencing of the neighbouring *MSH2* gene in Lynch syndrome patients. The underlying mechanism was found to involve *MSH2* promoter hypermethylation mediated by transcriptional read-through, thus providing an explanation of how colorectal cancer (CRC) susceptibility in these families could be inherited. Here we have investigated whether similar copy number variation (CNV)-based silencing mechanisms may underlie unexplained familial CRC cases.
METHODS: A genome-wide SNP array-based screen for constitutional CNVs was performed on a carefully selected cohort of microsatellite stable CRC patients without polyposis. Epigenetic silencing of one of the candidate genes showing CNV was established using allele-specific transcription and bisulfate sequencing.
RESULTS: We identified a constitutional duplication encompassing the 5' end of the *PTPRJ* gene, which was absent in >2,650 non-affected individuals. The duplication showed a head-to-tail in-tandem configuration. Transcriptional read-through and concomitant allele-specific silencing of the downstream *PTPRJ* locus by promoter hypermethylation was demonstarted in patient-derived tissues, but not in controls. Targeted screening of an independent cohort of unexplained familial CRC patients revealed a second case with again a partial, but not identical, *PTPRJ* duplication and a concomitant promoter hypermethylation. *PTPRJ* is a tumor suppressor gene that was previously identified as a CRC susceptibility gene in mice.
CONCLUSIONS: Our data indicate that *PTPRJ* may predispose to CRC through epigenetic gene silencing in humans, suggesting that CNV-based epimutations may serve as a more general mechanism in CRC predisposition.

P06.097

Research for new combinations of clinical and epigenetic markers significant in glioblastoma survival

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Glioblastoma (GBM) is the most common and malignant tumor of the Central Nervous System. GBM occur in 6.6 - 100 000 cases of Lithuania population per year. Also, GBM is the main cause of patient`s morbidity and high mortality rates. Due to infiltrative growth of those tumors the radical surgical removal becomes impossible. The prognosis for patients with diagnosed glioblastoma remains poor because of morphological and biological heterogenity determining inefficient effect of other types of treatment - chemotherapy and radiotherapy. GBM treatment results are still unsatisfactory. Therefore, the investigations of more precise and early diagnostic methods of these tumors become a topical issue.
Investigation on epigenetic alterations in glioblastomas is important for understanding of tumor biology as well as the disease pathogenesis for evaluation of early diagnostic possibilities and prognosis. In this study we investigate methylation of four epigenetic markers (MGMT, NDRG2, MLH, CASP8) involved in cell growth, DNA reparation and apoptosis, in glioblastoma tumor tissue, and select biomarkers significant in disease prognosis. Next, we investigate the relevance of set of clinical (patient age, gender, relapse free survival (RFS) and overall survival (OS)) and tumor characteristics (diameter, location) and epigenetic markers in glioblastoma patients prognosis.
All results we will present in a poster presentation.
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P06.098

Analysis of MMR system in human gliomas

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DNA mismatch repair (MMR) system defects result in an accumulation of mutations throughout the genome that can alter normal function of proteins and trigger tumorigenesis. We examined the MMR system in 27 low-grade astrocytomas, 19 anaplasic astrocytomas and 60 glioblastomas. MLH1, MSH2 and MSH6 protein expression was evaluated by immunohistochemical analysis, MMR genes methylation status by MS-MLPA, and microsatellite instability (MSI) of the tumors with 8 marker sequences. High-MSI was evident in 4.7% of gliomas, low-MSI in 38.4%, whereas MSS was found in 57%. More instable markers were BAT 40, MYCL1 and BAT25, instead of the frequently BAT26 instable marker related to colorectal cancer. MLH1, MSH2 and MSH6 protein expression was scored negative in 19.4%, 23.8% and 32.0% of cases. Furthermore, 9.3% of glial tumors showed loss of all of them and 43.3% had no expression of one or more MMR proteins. There was a correlation between MMR protein levels and grade of malignance. Tumors showed MLH1, MSH2 and MSH6 methylation in 80.7%, 49.1% and 49.1% of cases. We have not found fully association between methylation status, protein expression and microsatellite instability, suggesting that there could be other repair system which compensates the loss of MMR expression in MSS tumors and promotes MSI in other cases. The study of MLH1 promoter SNP (rs1800734) showed an association between AA genotype and loss of MLH1 expression. Finally, we also analyzed p53 protein expression. P53 overexpression was demonstrated in 46.5% of gliomas and it correlated with MLH1 gene methylation and MSH2 protein levels.

P06.099

Polymorphisms of the CYP1A1 and CYP2E1 genes in head and neck squamous cell carcinoma risk.

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Introduction: Polymorphisms in genes that encode P450 cytochrome enzymes may increase carcinogens activation or decrease their inactivation ability and consequently, developing cancer. The aims were identify the MspI-CYP1A1, PstI-CYP2E1 and DraI-CYP2E1 polymorphisms in patients with head and neck cancer (HNSCC) and compare with individuals without cancer; to evaluate the association of these polymorphisms with clinical-histophatological parameters.
Patients and Mehtods: In the case group, were evaluated 313 patients for CYP1A1, 217 for CYP2E1 (PstI) and 211 for CYP2E1 (DraI). In the control group were evaluated 417 individuals for CYP1A1, 334 for CYP2E1 (PstI) and 374 CYP2E1 (DraI). Molecular analysis was performed by PCR-RFLP technique and for statistical analysis were used chi-square and multiple logistic regression tests.
Results: We confirmed that age, smoking and alcohol consumption are risk factors for HNSCC. The Hardy-Weinberg analysis showed that genotypic frequencies are in equilibrium in the patients. For control group, the CYP2E1 (PstI) is in equilibrium and the others genes there were not in equilibrium. The result for CYP1A1 (MspI) showed that age (OR:8.15; CI 95% 5.57-11.92) and smoking (OR:5.37; CI 95% 3.52-8.21) were predictors for the disease; to the CYP2E1 (PstI and DraI), there were an association of age (PstI-OR:9.10; CI 95% 5.86-14.14/

DraI-OR:8.07 ; CI 95% 5.12-12.72), smoking (PstI-OR:4.10; CI 95% 2.44-6.89/ DraI-OR:5.73; CI 95% 3.34-9.82), alcohol (PstI-OR:1.93; CI 95% 1.18-3.16/ DraI-OR:1.69; CI 95% 1.02-2.81), respectively with disease development. CYP2E1 (PstI) was less frequent in patients group (OR:0.48; CI 95% 0.23-0.98). **Conclusion:** CYP2E1 PstI polymorphism may have a protective role in the disease.

P06.100
Association between 11 genetic polymorphisms in folate metabolizing genes and head and neck squamous cell carcinoma risk

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Introduction, Objectives and Methods: Polymorphisms in genes involved in metabolism folate may affect folate status due to their involvement in DNA methylation and synthesis and, therefore, the risk of head and neck cancer (HNSCC). We conducted a case-control study of 265 HNSCC cases and 466 non-cancer controls to investigate associations of *MTHFR* C677T and A1298C, *MTR* A2756G, *MTRR* A66G, *RFC1* A80G, *MTHFD1* G1958A, *CBS* 844ins68, *TC2* C776G and A67G, *SHMT* C1420T and *BHMT* G742A folate metabolism polymorphisms with HNSCC risk. Gene-environment interactions between polymorphisms and survival time, tobacco and alcohol habits, age and gender were evaluated by multiple logistic regression analysis. **Results:** We found that age ≥ 49 years (p<0.001), male gender (p=0.03), tobacco habit (p<0.001), *MTHFR* 1298AC/CC (P=0.028), *MTR* 2756AG/GG (P=0.010) and *RFC1* 80AG/GG (P=0.015) variant genotypes were associated with an increased risk of HNSCC. There were interactions between lower survival and *CBS* 844ins68 (P=0.005); age ≥ 49 years and *MTR* 2756 AG/GG (p=0.004) and *RFC1* 80AG/GG (p=0.006) genotypes; male gender and *MTHFR* 1298 AC/CC (p=0.030), *MTR* 2756 AG/GG (p=0.006) and *RFC1* 80 AG/GG (p=0.009) genotypes; tobacco non- habit and *MTHFD1* 1958GA/AA genotype (p=0.040); tobacco habit and *MTHFR* 1298 AC/CC (p=0.054) and *MTR* 2756 AG/GG (p=0.010); alcohol non- consume and *RFC1* 80 AG/GG (p=0.008) genotype with HNSCC increased risk. **Conclusions**: Our data provide evidence that folate metabolism genetic polymorphisms associated with variables as advanced age, male gender, tobacco and alcohol increase HNSCC development and *CBS* 844ins68 polymorphism is associated with less survival time.

P06.101
Genetic analysis of hematologic myeloid malignancies with acquired trisomy 21 as the sole cytogenetic change

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Although acquired trisomy 21 is one of the most common numerical abnormalities in acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and myeloproliferative neoplasms (MPN), little is known about its pathogenic impact or accompanying submicroscopic changes. Furthermore, previous studies have mainly focused on cases in which +21 was part of a complex karyotype. We performed single nucleotide polymorphism array and mutation analyses of the *FGFR1*, *FLT3*, *GATA1*, *JAK2*, *KIT*, *NPM1*, *NRAS*, *RUNX1*, and *TET2* genes on 11 myeloid malignancies with trisomy 21 as the sole cytogenetic aberration. Copy number alterations and uniparental disomies were observed in six (55%) cases, with none of the changes being recurrent. Mutations in *RUNX1*, *TET2*, and *NPM1* were detected in 5 (45%) cases. These results show that trisomy 21-positive myeloid malignancies are molecularly heterogeneous.

P06.102
Evolution of genomic instability in diethylnitrosamine-induced hepatocarcinogenesis in mice

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Diethylnitrosamine (DEN) is a hepatic procarcinogen which is frequently used as an inducer of hepatocellular carcinoma (HCC) in mice. Although mice after DEN exposure are among the most widely used models for liver tumorigenesis, a detailed, mechanistic characterization of the longitudinal changes in the respective tumor genomes has never been performed. We established the chronological order of genetic alterations during DEN carcinogenesis by examining mice at different points in time. Tumor samples were isolated by laser micro-dissection and subjected to array-comparative genomic hybridization (array-CGH) and sequencing analysis. Chromosomal gains and losses were already observed in tumors by week 32 and increased significantly by week 56. Loss of distal chromosome 4q, including the tumor suppressors *Runx3* and *Nr0b2/Shp*, was a frequent early event and persisted during all tumor stages. Surprisingly, sequencing revealed that β-catenin mutations occurred late and were clearly preceded by chromosomal instability. Thus, contrary to common belief, β-catenin mutations and activation of the Wnt/β-catenin pathway are not involved in tumor initiation in this model of chemical hepatocarcinogenesis. *Conclusion:* Our study suggests that the majority of the current knowledge about genomic changes in HCC is based on advanced tumor lesions and that systematic analyses of the chronologic order including early lesions may reveal new, unexpected findings.

P06.103
Type II MHC loci predispose hepatocellular carcinoma in a genome-wide association study of Hong Kong Chinese

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Hepatocellular carcinoma (HCC) is one of the world's leading causes of cancer-related death, especially in East Asia countries. Environmental factors had been identified for the development of HCC, such as chronic infection with heptatitis B virus (HBV) in Asian populations. Although there is a high degree of familial segregation of HCC cases, the genetic risk factors of HCC development are little understood. We are one of the first to perform a genome-wide association study (GWAS) of Chinese HCC patients in this study. The study cohort consisted of 300 Hong Kong Chinese male patients who were all positive for HBV surface antigen (HBsAg). Using the allele distribution of a Chinese population control sample, we detected four most significant hits on chromosome 6 (P-value ≤ 5.0×10^{-6}). These 4 SNPs, located in the major histocompatibility complex (MHC) region around the HLA-DQB2 and HLA-DPB2 gene loci, were significant even after correction for population structure. This result supported previous findings that HLA loci have a putative role in the clearance of HBV virus and the progression to chronic liver disease among HBV carriers. Haplotype analysis is being performed and suggests that certain type II alleles are associated with HCC.

P06.104
The BRCA1 variants c.692C>T and c.693G>A affect a putative ESE motif and increase exon 11 skipping

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BRCA1 variants c.692C>T (p.T231M) and c.693G>A (p.=), of unknown clinical significance, were identified in two different patients from HBOC families. The variants lie in exon 11, within a region previously described as "critical region", which spans between codons 200-300. Others suggested that this region was an important regulatory region and suggested the presence of two ESE motifs, one of these covering positions c.690 to c.695. We assessed the putative effect of these variants on RNA splicing.

For splicing analysis, lymphocytes from the carriers and healthy controls were cultured and a fraction of these was treated with puromycin to prevent nonsense-mRNA mediated decay. The relative contribution of each allele to different transcripts was assessed based on allele specific cDNA analysis. In addition, an exon trapping vector

including the putative ESE motif will be used to show its functionality in presence or absence of the variants.
The alleles with either variant give rise to normal levels of the full-length transcript, but also induce higher expression of in-frame *BRCA1*Δ11 isoform, which was found to be weakly expressed in controls. Results of the ESE-dependent splicing assay will be reported.
We now provide experimental evidence for the presence of a functional ESE motif in exon 11. Both variants in this motif affect splicing by increasing the expression of the *BRCA1*Δ11 transcript, besides expressing the full-length transcript. Currently, the clinical relevance of these variants remains elusive, as both the function and the critical expression level of the *BRCA1*Δ11 transcript are unknown.

P06.105
RAD51C - a novel predisposing gene for breast and ovarian cancer: validation of other RAD51 paralogs and missense mutations

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Even high risk families for breast and ovarian cancer families can only partially be explained by mutations in the known highly penetrant genes *BRCA1* and *BRCA2*. Therefore, extreme genetic heterogeneity serves as a model for inherited predis-position to breast cancer. In agreement with this hypothesis, we have recently identified a novel high penetrant predisposing gene for breast and ovarian cancer. The gene encoding RAD51C, one of five known RAD51 paralogs, has been found mutated in six out of 480 breast- and ovarian cancer (BC/OC) families (1.3%). In addition, we have identified a more common missense mutation (2%), exhibiting an OR of 3.44 in BC/OC families only (c.790G->A; G264S).
In order to test whether other RAD51 paralogs are mutated in BC/OC or BC families, we now screened the genes encoding RAD51B and XRCC3, respectively. Apart from one and two misense mutations, respectively, no mutations were found in the *RAD51B* and *XRCC3* genes. Although the application of several algorithms is indicating pathogeneity of the identified missense aberrations, mutations in the other RAD51 paralogs seem therefore to be rare.
To validate further missense mutations identified in the *RAD51C* gene, we employed different functional assays. E. g. the functional characterization of novel missense mutations by a transfection assay found in the *RAD51C* gene led to the identification of at least five further pathogenic mutations in the *RAD51C* gene and will also be reported.
In summary, our data confirm RAD51C as a novel predisposing gene for BC/OC, but might exclude other genes encoding RAD51 paralogs.

P06.106
Predominant RET germline mutations in exons 10, 11, and 16 in Iranian patients with hereditary medullary thyroid carcinoma

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Introduction: Medullary thyroid carcinoma occurs in both sporadic (75%) and hereditary (25%) forms. The missense gain-of-function mutations of the RET proto-oncogene in MTC development has been well demonstrated.
The aim of this study was to investigate the spectrum of predominant RET germline mutations in exons 10, 11, and 16 among Iranian hereditary MTC patients.
Materials & Methods: In this study 217 participants (151 MTC patients and 66 their first-degree relatives) were included. Genomic DNAs were extracted from the leukocytes according to the standard Salting Out/Proteinase K method. Mutation detection was performed through PCR-RFLP analysis. For Positive patients direct DNA sequencing was carried out.
Results: In 217 participants, 43 missense mutations were identified in exons 10(6%, 13 of 217), 11(13%, 28 of 217) and 16(0.9%, 2 of 217). Moreover, a novel germline mutation was detected at codon 686 in exon 11(S686N). Also four different polymorphisms were found in intron 16 of the RET proto-oncogene in eight patients that most of them carried another mutation in exons 10 or 11.
Conclusion: The obtained data in this study showed the frequency profile of the RET proto-oncogene mutations in a sample of 217 Iranian individuals with MTC (19.8%). The most frequent mutation in our population was C634G; whereas in most population it was C634R. Therefore the transforming activity and functional effect(s) of a new RET mutants such as S686N and intronic polymorphisms remain to be elucidated. Altogether, these results underline the importance of the genetic background of family members of any patient with MTC.

P06.107
Methylation of proximal MLH1 promoter region is a sensitive and specific molecular method to distinguish HNPCC from sporadic CRCs

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Hereditary Nonpolyposis Colorectal Cancer (HNPCC) is an inherited colorectal cancer syndrome and accounts for 5% of all colorectal cancer (CRC). A significant breakthrough in the diagnosis of HNPCC was made with the discovery of mismatch repair (MMR) genes that cause HNPCC. An alternative method for identifying HNPCC patients is microsatellite instability (MSI). In sporadic CRCs, inactivation of MLH1 gene by promoter methylation causes MSI. Significant differences in the clinical phenotype between HNPCC families from South Korea and from the Netherlands were observed.
To investigate methylation status in both distal and proximal regions of MLH1 gene, we used methylation-specific PCR (MSP) and quantitative real-time PCR (MethyLight) using 14 HNPCC and 37 sporadic MSI-H CRCs. Germline mutations for MMR genes were tested in HNPCC patients, and BRAF V600E mutation and MLH1 protein expressions were analyzed in all CRCs.
Germline mutations of MLH1 (n=7) and MSH2 (n=3) were detected in 10 of 14 HNPCC patients. BRAF mutations were detected in 2 (5.4%) sporadic CRCs.
In CRCs from HNPCC patients, proximal and distal promoter regions of MLH1 gene was methylated 0% and 28.6% of cases, respectively. In sporadic MSI-high CRCs, 62.2% and 67.6% were methylated in the proximal and distal promoter regions of MLH1 gene. After combining both proximal and distal regions of MLH1 promoter, methylation frequencies increased up to 82%.
In MSI-H CRCs, methylation test using proximal MLH1 promoter region is an important molecular method to distinguish HNPCC from sporadic CRCs with high sensitivity and specificity.

P06.108
Epidemiology of HPV infection and possible polymorphisms associated with cervical cancer in Azores - Portugal.

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Cervical cancer (CC) is the third most common cancer in women worldwide, and the seventh overall, with an estimated 530 000 new cases and 275 000 deaths in 2008. Human papillomavirus (HPV) is mainly responsible for this disease and HPV16 and HPV18 are the most important oncogenic types.
The genetic factors that contribute to the development of CC have not yet been fully identified but numerous studies suggest that the immune response is preponderant in the success or failure of the HPV infection and subsequent progression to cancer.
In this study we sought to identify and genotype HPV in CC in the Azorean population. We also tried to investigate the possible

association between this disease and polymorphisms chosen from 5 genes involved in the immune response to CC.
A total of 78 paraffin sections, with tissue samples of CC were retrospectively evaluated between 1992 and 2008. After viral and genomic DNA extraction we proceeded to HPV genotyping, and identification of six polymorphisms in the TP35 , MTHFR, IL23R, IL12A and IL12B genes.
The most frequently detected HPV type was HPV16 followed by HPV58 and HPV31. The less frequent types were HPV18, HPV33, HPV45, HPV35, HPV51 and HPV52. Regarding genetic susceptibility factors, the most common variants identified in patients were, the variant GG in TP53, CC in MTHFR, CC in IL-23R Rs10889677 and GT in Rs7517847, GG in IL-12A and variant AA in IL-12B. No statistically significant association was identified between the genetic polymorphisms and progression to CC.

P06.109
SESN3-dependent regulation of 2-Cys Prx by HSF1

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Induction of heat shock response is accompanied by elevated transcription of Heat Shock Proteins, which are controlled by HSF1. Under normal conditions HSF1 appears as a repressed monomer shuttling between cytoplasm and nucleus. Upon stress HSF1 becomes activated which is associated with trimerization, nuclear accumulation, hyperphosphorylation, acquisition of DNA-binding and transactivation capacity. HSF1 protects cells from various stresses and increases cellular viability. It can also promote tumor survival as cancer cells depleted in HSF1 show significantly reduced growth and survival. Interestingly, HSF1 regulates up to 3% of genome beside HSPs and regulation mechanisms differ between tumor and normal cells. HSF1 role in the regulation of genes beside HSPs and their link to tumorigenesis has not been well studied.
We found that promoters of sestrin family genes contained heat shock elements. We assumed that HSF1 may influence peroxiredoxins' activity and subsequently regulate the level of Reactive Oxygen Species via regulation of sestrins.
Our results show that introduction of constitutively-active HSF1 into carcinoma cells increases the level of ROS and decreases the level of SESN3 mRNA. Surprisingly, introduction of active HSF1 into normal fibroblasts causes opposite effects. Active HSF1 slightly decreases tumor cell growth and increases cell growth of normal cells. According to our study HSF1 influences the activity of 2-Cys-Prx and this effect seems to be SESN3-dependent as it was proved by shRNA analysis. We believe that our study will contribute to our better understanding of HSF1 role in the regulation of pathways beside HSP induction and subsequently in tumorigenesis.

P06.110
Global methylation levels in oral potentially malignant disorders

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Background: Epigenetic alterations are common events in human cancer. Tumor cells show an aberrant reduction of global methylation levels and aberrant increase of gene-specific methylation. In this regard, global hypomethylation seems to be a significant factor driving oncogenesis. However, so far, little is known about the global methylation levels in common potentially malignant disorders such as oral lichenoid disease (OLD) and oral leucoplakia (OL), which malignant potential is controversial. The aim of our study was to assess the degree of global genomic hypomethylation in oral scrapings of patients with oral potentially malignant disorders compared to normal patients.
Methods: We analyzed 205 cytological samples; 153 OLD patients, 22 OL patients and 30 controls. DNA was extracted from the oral scraping and the global methylation level was asses by analyzing the methylation of repetitive elements (LINE1) by pyrosequencing after bisulphite treatment.
Results: OLD and LO samples displayed a mean global methylation level of 65.64% (SD=2.46) and 65.56% (SD=1.36) respectively. The reduction of the methylation level in both groups was statistically significant (p=0.002 and p=0.005) when compared to the control group. About 37.9% of OLD and 45.5% of LO samples were globally hypomethylated when evaluated against the minimum level of methylation in control samples.
Conclusion: In oral malignant disorders the presence of global hypomethylation may indicate that carcinogenic processes could be taking place and may favour the development additional alterations.
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P06.111
Association of Inflammatory Pathway Genes Polymorphism in Genetic Predisposition to Prostate Cancer Risk: A Pilot Study from Northern India

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Background: Prostate cancer (PCa) is the most frequently diagnosed cancer and one of the major causes of death in men. An understanding of factors associated with PCa mortality is increasingly important. Accumulating evidence implicates inflammation in PCa. Genetic variations within the sequence of candidate inflammatory genes like *TLRs* 2, 3, 9 and *COX-2* genes may lead to suboptimal inflammatory capacity and therefore increased PCa risk.
Material and Methods: We genotyped 195 PCa and 250 healthy controls for *TLR2* (−196 to −174 Del), *TLR3* (c.1377C/T) [rs3775290], *TLR9* (G2848A) [rs352140], and *COX-2* (-765 G>C) [rs20417], (+8473 T>C) [rs5275] promoter gene polymorphisms using PCR-RFLP.
Results: Variant allele carrier of *TLR2* (ID+DD) and *COX-2* -765 (GC+CC) demonstrated increased risk (p= 0.007; OR=2.00; p=0.016; OR=1.74) for PCa. Similarly (TC+CC) of *COX-2* +8473 also exhibited borderline risk. Variant genotype CC of *COX-2* +8473 was found to be significantly associated with the overall higher risk of PCa (p=0.045; OR=1.82). The diplotype C-C of *COX-2* was observed to be associated with a significant increased PCa risk (p=0.004; OR=4.26). Stratification of cases based on clinical pathological grade only combined genotype (CT+TT) of *TLR3* demonstrated borderline significant (p=0.051; OR=2.14) conferring a marginal increased risk with high Gleason grade of PCa patients.
Conclusion: Our findings confirm that the *TLR2*, *COX-2* polymorphism and diplotype of *COX-2* could be a risk factor for susceptibility of PCa. Further validations in large population-based studies are needed to elucidate the role of these SNPs for PCa risk.

P06.112
Association of -607 C/A polymorphism of Interleukin-18 with breast cancer in Zahedan Southeast Iran

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OBJECTIVE: Interleukin-18 (IL-18) is a multifunctional cytokine that induces the production of interferon-γ (IFN-γ) and play a key role in antitumor immunity. The aim of the present study was to determine whether the -607 C/A polymorphism of IL-18 is associated with breast cancer susceptibility in a sample of Iranian women.
MATERIAL AND METHODS: This case-control study was performed on 74 women with breast cancer and 93 healthy women. Genomic DNA from patients and control subjects were extracted from peripheral blood using salting out, and IL-18 gene promoter polymorphismat position -607 C/A was determined by tetraprimer-ARMS PCR.
RESULTS: No significant difference was found regarding -607 C/A polymorphism of IL-18 among case and control groups ($\chi 2=1.89$, P=0.389).
CONCLUSION: Our results indicate that -607 C/A polymorphism of IL-18 is not a risk factor for breast cancer in the southeastern Iranian subjects.

P06.113
The insulin receptor substrate 4 (*IRS4*) gene is mutated in paediatric T-cell acute lymphoblastic leukaemia

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We recently reported that the t(X;7)(q22;q34) in a paediatric T-cell acute lymphoblastic leukaemia (T-ALL) results in overexpression of the insulin receptor substrate 4 (*IRS4*) gene through illegitimate recombination with the T-cell receptor ß locus, and hypothesised that *IRS4* may be activated through other mechanisms in additional cases, akin to what has been shown for *NOTCH1*. The *IRS4* gene consists of only one exon, which was sequenced in 21 paediatric T-cell ALL cases. Apart from three known polymorphisms in four cases, two (10%) were shown to harbour a mutation - a 594 bp deletion and a missense mutation, respectively. The former was an interstitial in-frame deletion depriving the protein of the pleckstrin homology domain, a well-conserved region important for the function of the IRS family, whereas the latter was shown to be acquired and to replace a proline with a threonine. We conclude that *IRS4* is mutated in a proportion of T-ALL. However, the pathogenetic impact and clinical ramification of such mutations remain to be elucidated.

P06.114
The JAK2 V617F mutation incidence in patients with myeloproliferative neoplasms.

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A point mutation V617F in the *JAK2* gene occurs in nearly all patients with polycythaemia vera (PF) and in other myeloproliferative neoplasms like essentail thrombocythemia (ET) and myelofibrosis (MF). The *JAK2* V617F mutation was study in 491 patients with diagnosis or suspect of myeloproliferative neoplasms. In nearly 80% of patients mutation was detected. Althought discover of *JAK2* V617F mutation has provided important insight into the molecular etiology of PV, ET and MF there is still frequent set of patients without this mutation. Further study of genetic events in pathogenesis in myeloproliferative neoplasms are necessary to evaluate prognosis especially for patients without *JAK2* V617F mutation.

Frequency of V617F JAK2 mutation in patients with myeloproliferative neoplasms.

Diagnosis	No of patients	No of patients with homozygotic *JAK2* V617F mutation	No of patients with heterozygotic *JAK2* V617F mutation	No of patients with without *JAK2* V617F mutation
Essential thrombocythaemia	216	0 (0%)	133 (62%)	83 (38%)
Polycythaemia vera	132	20 (15%)	86 (65%)	26 (20%)
Nonclassified myeloproliferative disorders	82	4 (5%)	47 (57%)	31 (38%)
Essential myelofibrosis	16	2 (12%)	6 (38%)	8 (50%)
Chronic myelogenous leukemia	15	1 (7%)	2 (13%)	12 (80%)
Together	461	**27 (5,8%)**	**274 (59%)**	**160 (35,2%)**

P06.115
Chromosomal study among CML patients

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Many different chromosome aberrations have been shown in cancers. For the first time, Dr. Janet Rowley, American geneticist, discovered a translocation of 22q to another chromosome, generally 9q, in the bone marrow cells of more than 90% of patients with chronic myelogenous leukemia (CML), which called the Philadelphia chromosome. On the other hand, many malignancies exhibit multiple chromosome abnormalities.

The aim of this study was to investigate chromosome aberrations among the CML patients. During the last four years a total of 94 patients were referred to our Department from different hospitals with the primary diagnosis of CML. These patients consisted of 46 men(48.9%) and 48 women (51.1%). Specific technique for the bone marrow culture and also the G-banding technique for karyotyping were applied for all the samples.

Out of 94 cases, 44 patients (46.8%), consisted of 20 males (21.3%) and 24 females (25.5%), showed normal karyotypes.

50 patients (53.2%) showed abnormal karyotypes. 33 patients (35.1%) revealed Philadelphia positive, consisted of 17 males (18.1%) and 16 females (17%).

We observed different numerical aberrations among 8 (8.5%) patients (5 males and 3 females). We also found structural aberrations among 7 (7.4%) patients (5 males and 2 females). The results were obtained as follows:

t(2;5) (one male), inv(16) (one male), del5p (one male), t(1;3;13;18) (one male), t(2;3;14;17) (one male), t(8;21) (one female). t(1;12) (one female).

P06.116
Genotyping KRAS and BRAF mutations in paraffin-embedded cancer research samples

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The epidermal growth factor receptor (EGFR) pathway is a complex signalling cascade that is associated with the development and progression of many cancer conditions. It has been shown that approximately 35-45% of metastatic colorectal cancer tumors may have a KRAS or BRAF mutation, which makes them less likely to respond to anti-EGFR therapies. The identification of these mutations is therefore of great importance in clinical and pharmaceutical research. The Applied Biosystems® KRAS Mutation Analysis Reagents and BRAF Mutation Analysis Reagents were developed to facilitate research aimed at elucidating the role of these regulatory proteins in oncology. The reagents offer a simple protocol for the detection of 12 mutations in the KRAS gene and 3 mutations in the BRAF gene. In this collaborative research study, we made use of the Applied Biosystems® KRAS and BRAF Mutation Analysis Reagents and the Applied Biosystems 3500 Dx, 3100 and 310 Genetic Analyzers to genotype known variants on 35 cancer research samples. The KRAS and BRAF Mutation Analysis Reagents results were easy to interpret and equivalent between the Applied Biosystems® 3100 and 310 Genetic Analyzers. The 3500 Dx instrument technology may also ease the analysis and create standardization for genetics laboratories, which will help alleviate the different quality of results that stem from varying technologies and chemistries.The Applied Biosystems® KRAS and BRAF Mutation Analysis Reagents are For Research Use Only. Not for use in diagnostic procedures

P06.117
Accurate and Sensitive Detection of KRAS Mutations in Heterogeneous Cancer Specimens

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The discovery of pivotal genetic alterations and the understanding of their role in cancer is leading to remarkable successes in therapeutics

and patient care. Molecular diagnosis methods such as DNA sequencing and conventional genotyping of tumor biopsies have advanced research in this field, but are limited in sensitivity due to stromal contamination and by genetic heterogeneity in cancer. We have developed competitive allele specific TaqMan® PCR (castPCR) assays assays for detecting cancer-associated sequence variations. CastPCR not only maintains the wide dynamic range, high sensitivity and reproducibility of TaqMan® assays but also greatly improves the specificity. The technology enables detection, of as little as 1 mutant allele molecule in 10,000,000 wild type molecules. We report here sensitive and accurate detection of cancer-associated KRAS mutations within formalin-fixed paraffin-embedded (FFPE) heterogeneous cancer specimens. Eight FFPE model cell lines were initially used to validate the assays. Mutant FFPE cell line DNAs were titrated in the FFPE wild type cell line DNAs from 100% to 0.1%. Mutations were easily identified at the level of 0.1% with high reproducibility. 24 anonymous tumor tissues and 12 non-tumor tissues from FFPE specimens were also examined. No positive samples were found in non-tumor tissues. The results obtained by castPCR for the 24 tumor tissues were concordant to those previously reported by three different methods (Taqman® PCR, Taqman® PCR + PNA and Sequencing). Our results demonstrate that castPCR as a new rare mutation detection technology, has greater sensitivity, specificity and can thereby facilitate accurate molecular diagnosis of heterogeneous cancer specimens.

P06.118
Detection of K-RAS point mutations in colon tumors by PCR-RFLP method

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The prognosis of patients with metastatic colorectal carcinomas (CRC) was improved by the new anti-Epidermal Growth Factor Receptor (EGFR) therapy. KRAS mutation status is a crucial parameter for selecting patients for anti-EGFR therapy, only patients without mutant tumors benefit from the new therapy. Gain-of-function K-RAS point mutations, present in 25-45% CRC, maintain the active form of the K-RAS protein, leading to EGFR-independent activation of intracellular Ras/Raf/mitogen-activated protein kinase (MAPK) signaling pathways, making the anti-EGFR tumor therapy ineffective.

The aim of our study was to detect K-RAS mutation status in codons 12 and 13 (about 99 % of all mutations in the K-RAS gene) in CRC patients in order to contribute to the selection of patients for applying the new therapy with Avastin (an anti-EGFR agent) in Romania.

DNA was isolated from fixed and paraffin embedded primary and/or metastatic tumors from 169 CRC patients. K-RAS mutation status was analyzed by the PCR-Restriction Fragment Length Polymorphism (RFLP) method optimized in our laboratory.

Up to now, gain-of-function mutation was detected in 62 patients (36,69%), of which 58 in codon 12 and 4 in codon 13.

The international validation of our PCR-RFLP method by successfully participating to the "Ring trial Molecular-Pathological KRAS Mutationtest of Colorectal Carcinoma" (organized by the German Society of Pathology and the German Federal Association of Pathologists) and to the EQA testing has offered the opportunity to assess the K-RAS mutation status in our laboratory by the low cost and the European analysis standard.

P06.119
K-ras exon1mutations in colorectal cancer patients and its association with clinicopathological information

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Colorectal cancer (CRC) is the most gastrointestinal cancer in United States and Europe. It is the third most common cancer in Iranian men, and fourth in Iranian women. According to recent researches, somatic mutations in codons 12, 13 (exon1) of K-ras gene are discovered in 20% - 50% human CRCs. The aim of this study was to estimate the contribution of K-ras gene mutations in codons 12, 13 in the incidence, and its association with clinicopathologic information like age, sex, familial history, site of primary and histology in Iranian Colorectal cancer patients. We have analyzed 59 tissue specimens of colorectal cancer patients using PCR/sequencing method for codons 12, 13 of K-ras gene. 20.3% of patients (10 in codon 12 and 2 in codon 13) have shown a point mutation. About 60% of mutations occur in rectum and 41.7% in colon. More than 80% of mutations were in adenocarcinomas and less mutations in mucinous. Most mutations were found at the age of 60 and more. Only two of patients had a familial history for cancer. Mutations in codons 12, 13 are not common in Iranian patients. The mutation pattern for Iranian patients differs from other nationalities. Perhaps we can find point mutations in other exons, and we suggest whole genome sequencing for our patients.

P06.120
Spectrum of kras mutations in colorectal cancer: adenoma vs. adenocarcinoma

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Introduction: The evolution of colorectal cancer from polyps was approved. It was recognized that most colorectal cancers arise in a process progressing from adenomas to carcinomas. Kras, a small intracellular GTPase, is a central intermediary of the epidermal growth factor receptor (EGFR) pathways. Kras mutations can be detected in approximately 30-60% of patients with colorectal cancer. The most hot spot of the gene is located in exons 2 and 3. The aim of the current investigation was to examine profile of Kras mutations in adenomas and carcinoma in these exons.

Methods: In this study exon 2 and 3 Kras gene in 48 tumors and 47 polyps biopsy were analyzed. DNA of the specimens were scanned using PCR sequencing.

Results: Six mutations in tumors including 5 mutations in codon 12 (3 Gly12Asp ,1 Gly12Ala and 1Gly12Gly) and 1 mutation in codon 13, Gly13 Asp, were detected. Moreover, 3 mutations determined in polyps including 2 in codon12, Gly12Asp, and one in codon 13 ,Gly13 Cys.

Conclusion: Our findings showed that probably the profile of mutations in adenocarcinoma is not entirely compatible with the pattern of mutations in adenomas. However, just one of the mutations,Gly12Asp, was similar in the both group. Therefore, it seems that scanning the mutation in young individuals with polyps will be helpful for early diagnosis clinically and genetically and it would be useful for other follow up.

Key words: Kras gene, Colorectal cancer, Mutation, adenoma , adenocarcinoma.

P06.121
Frequency and type of KRAS mutation in Turkish colorectal carcinoma population

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Molecular-targeted agents by blocking epidermal growth factor receptor improve survival in patients with metastatic colorectal carcinoma (mCRC). But anti-EGFR therapy is only applicable to a subset of patients. It has become clear that mutations in intracellular signaling pathway negatively predict success of anti-EGFR therapies. In the intracellular signaling cascade, gain of function mutation in KRAS gene is the most documented one.

We aimed to analyze the frequency and type of KRAS mutation and its correlation with other clinicopathological parameters which was not well documented so far in Turkish mCRC patients.

A hundred and twenty three cases of mCRC were screened for KRAS mutational status of codon 12 and 13 of the KRAS by using Real-time based assay. The correlations with KRAS mutation status and clinicopathological parameters were analyzed statistically.

Male/female was 75/48 and mean age was 60.64. KRAS mutation was found in 53 (43.1%) cases. The most common mutation type was

12 ASP (33.3%) and second was 12VAL (26.3%). 13ASP mutation was approved in 17.5% of cases. The frequency of KRAS mutation in mucinous carcinomas was higher than in adenocarcinomas. Most common mutation type was 13ASP in mucinous carcinomas. There was not significant correlation between KRAS mutation and tumor grade, Lymph node metastases and perineural invasion.
The frequency and type of KRAS mutation in Turkey showed similarity with the other studies. Mucinous tumors have poor prognosis since they have tendency towards higher KRAS mutation. More detailed studies questioning the association between KRAS mutation and histological subtypes are necessary.

P06.122
Promoter hypermethylation of DNA repair genes in Bulgarian patients with laryngeal carcinoma

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Background: Promoter hypermethylation is a central mechanism for epigenetic inactivation by which key genes are silenced in cancer cells. Genes that are frequently methylated in laryngeal tumours are involved in cell cycle control, DNA damage repair, apoptosis and tumour cell invasion. The aim of the current study was to examine promoter hypermethylation in two highly methylated in laryngeal carcinoma genes - *MGMT* and *MLH1*. They are both involved in DNA repair and are also associated with chemotherapeutic sensitivity.
Methods: DNA was extracted from fresh frozen tumour tissues of 50 patients with laryngeal cancer. After sodium bisulfite conversion, promoter regions of *MGMT* and *MLH1* were each amplified by PCR using two sets of primers - specific for methylated and unmethylated DNA.
Results: MGMT promoter was found to be methylated in 10 (20%) patients, while hypermethylation in *MLH1* was detected in 34 (68%) tumours. Epigenetic changes in *MGMT* were more frequent in older patients (p=0.02). No methylation of *MGMT* was observed in patients with early stage (T1-T2) carcinoma of the larynx. Hypermethylation of *MLH1* was significantly increased in the group of active smokers with excess alcohol consumption (p=0.04).
Conclusion: Epigenetic inactivation of DNA repair genes *MGMT* and *MLH1* plays a significant role in laryngeal carcinogenesis of Bulgarian patients.

P06.123
Global hypomethylation and expression level of the DNA methyltransferses in patients with laryngeal cancres - preliminary results.

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Laryngeal cancer is one of the most frequent head and neck cancers (HNSCC, Head and Neck Squamous Cell Carcinoma). About 95% of laryngeal cancers are squamous cell carcinomas (LSCC). The aetiology of LSCC is very complex, with both environmental (alcohol and tobacco use), genetic and epigenetic factors involved. One of the common epigenetic events, which has been recognized as a cause of carcinogenesis in a variety of tumors, is global DNA hypomethylation, namely reduced level of methylation compared with healthy tissue. Hypomethylation affects many repetitive sequences as well as a number of single-copy genes (e.g. growth regulatory genes, tissue specific genes) and may lead to chromosomal instability, loss of imprinting, retrotransposons and oncogene activation. DNA hypomethylation may occur at least partly as a consequence of cell cycle deregulation disturbing the coordination between DNA replication and activity of DNA methyltransferases.
The aim of study was 1) to assess the overall level of 5-methyldeoxycytosine in dinucleotide CpG sites in the genome of primary LSCC compared to normal laryngeal tissue; and 2) to examine of the expression level of the methyltransferases (Dntm1, Dntm3A, Dntm3B).
The study was carried out on a homogenous group of 70 patients with laryngeal squamous cell carcinoma.
Analysis of hypomethylation was performed using Ultra Performance Liquid Chromatography (UPLC) method. To analyze expression of panel genes, Real−Time PCR was used. We compare the results of the global level of DNA methylation and the activity of DNA methyltransferases with clinical and histopatological data as well tobacco and alcohol consumption.

P06.124
Investigation of mutations in ABL and BCR gene in *BCR/ABL* transcript of patients with ALL and CML

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Chronic Myelogenous Leukemia (CML) is characterized as a presence of the *BCR/ABL* gene fusion or Ph chromosome. The breakpoints can be changed on BCR and ABL genes and resulting in different molecular weights of the fusion gene products. Different size deletions and mutations are seen in ABL and BCR genes along with the presence of the *BCR/ABL* fusion gene. These mutations are associated with patient's prognosis and related to therapy. In the present study, F317L, F311I, M351T, T315I and F359V mutations was analized in ALL and CML patients with *BCR/ABL* fusion gene and control cell line K562 having *BCR/ABL* fusion gene and Raji (Burkitt lymphoma) cell lines without *BCR/ABL* fusion gene on *BCR/ABL* transcript (cDNA), by using PCR-RFLP technique.
Fourteen out of 67 patients showed these five type mutations either alone or along with others. These mutations include F317L in 7 patients, F311I in 3 patients, M351T in 2 patients, T315I in one patient and F359V mutations in 3 patients and also M351T and F317L in one patient, T315I and F317L in the other patient. As a conclusion, in order to determination of certain mutations in the *BCR/ABL* fusion gene is important for treatment and follow up treatment response.
Key Words: Leukemia; *BCR/ABL* , PCR-RFLP
This study was supported by Selçuk University Research Fund (No:10202019)

P06.125
Micro RNA expression profiling in childhood acute leukemia by microarray platform

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The aim of this study is to evaluate the associations between miRNAs and childhood acute leukemias to find out their clinical and therapeutic implications. Sixty children with newly diagnosed acute childhood leukemia and 15 age-matched controls were included in the study. Follow-up period was between 9 and 18 months. During this period, two patients relapsed and 7 patients died. MicroRNA expression profiling consisting of 1136 miRNAs from peripheral blood (PB) and bone marrow (BM) samples of patients and control cases were analysed. Aberrant microRNA expressions associated with the diagnosis, differential diagnosis, outcome and prognosis of acute leukemia were prospectively evaluated. The effect of significant miRNAs on overall and event free survival for ALL and AML separately were also presented. The results were confirmed by real time RT-PCR. PB miRNA profile does not reflect BM miRNA profile. Significant dysregulation according to the BM results compared to the control group was found in ALL, AML, high risk ALL and AML, T-cell and B-cell ALL, and exitus groups . miR-548i, miR-640, miR-606, miR-3140, miR-3115, miR-369-3p, miR-574-3p, miR-613 and miR-140-3p are described for the first time in leukemia. miR-145 for ALL and miR 140-3p for AML were associated with overall survival. Novel miRNAs which are associated with childhood acute leukemia were identified. In

conclusion, miRNA expression profiles may be a powerful tool for the diagnosis and differential diagnosis of acute leukemia and may have a prognostic significance.
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P06.126
HTFA (High - Throughput FISH Analysis): A new sensitive approach to screen hematological malignancies

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INTRODUCTION: Multiple probes developed by using bacterial artificial chromosome (BAC) for the diagnosis of hematologic malignancies are new diagnostic tools facilitating the detection and mapping of genomic alterations in target genes. This method helps screening of several deletion-duplication regions unlike conventional techniques and it provides a hematologic approach. High-Throughput FISH Analysis platforms contain 31 regions of somatic chromosomes related to hematologic malignancies.
MATERIALS and METHOD: 20 cases diagnosed as leukemia from Kocaeli University Faculty of Medicine Hematology Policlinic were analysed in this study. DNA samples which labelled according to the protocol were hybridized with High-Throughput FISH Analysis platforms and they were screened and analysed with the help of BlueFuse Multi v2.1 software.
RESULTS: According to the data given, deletion was detected in one patient with the size of 55,414,419.5 bp between the bands 5(q21.2)-q33.3). 3 deletions were detected in another patient at the following bands: between the bands 7(p22.3-q31.3) with the size of 118,801,746.5 bp, between the bands 7(q31.3-q35) with the size of 17,543,770.0 bp, between the bands 7(q35-q36.3) with the size of 11,622,058.0 bp. 1 duplication was detected in one patient with the size of 558,808.5 bp on the band 3q11.2.
CONCLUSION: The results of three cases were used in the differential diagnosis of AML-MDS by the Department of Clinical Hematology. Diagnosis and treatment of two patients were given according to the results we provided. Preliminary findings showing that BAC based HTFA are effectively applicable in the diagnosis of hematological malignancies.

P06.127***
Detection, using custom-designed CGH microarray, of rare CNVs in Li-Fraumeni patients without *TP53* mutations

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Li-Fraumeni syndrome (LFS), resulting from mutations of the *TP53* tumor suppressor gene, is a devastating inherited form of cancers, characterized by an early onset and a broad spectrum of tumors. We have identified, thanks to a national recruitment of more than 1000 families suggestive of LFS, *TP53* alteration in 156 affected families. To estimate the contribution of deleterious CNVs (*Copy Number Variations*) to LFS in families without detectable *TP53* alteration, we have developed a custom-designed CGH microarray. This microarray contains 24000 probes at a very high density covering 400 kb around the *TP53* locus and different genes belonging to the p53 pathway, and 150000 probes covering the rest of the genome. The analysis with this microarray of 30 families strongly suggestive of LFS, without detectable *TP53* mutation, allowed us (i) to characterize for the first time an intragenic deleterious duplication of *TP53*, (ii) to show the absence of quantitative alterations affecting the 5' and 3' regulatory regions of *TP53,* and (iii) to detect 9 singleton CNVs not recorded in the Database of Genomic Variants. Targeted analysis of these CNVs, using QMPSF, in 500 controls revealed that one CNV covering a miRNA was in fact polymorphic and that the 8 others were exclusive of LFS. Remarkably, 3 of these CNVs alter genes involved in the p53 pathway.

P06.128
Quantitative allelic imbalance(s) at the LNCR2 region (15q) in Czech patients with nonsmall cell lung cancer (NSCLC)

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Genes for nicotinic acetylcholine receptors (nAChRs) are members of region on chromosome 15q24-25.1 known as a lung cancer susceptibly locus 2 (LNCR2). We have introduced the SNaPshot analysis of four SNPs in the vicinity of the CHRNA5 and CHRNA3 genes to look for association between haplotypes in this region and the diagnosis of (or even the predispositition to) lung cancer in Czech population. First, we followed these four SNPs in genomic DNA samples of three groups of patients (with NSCLC, with non-neoplastic pulmonary diseases, and anonymized individuals from routine laboratory, respectively). The highest difference in genotypic frequencies was seen between the groups of NSCLC patients and control subjets with non-neoplastic pulmonary diseases (P = 6.3 x 10-5 for rs1051730 and P = 7.9 x 10-5 for rs8034191). Further, we applied this analysis to a set of 47 pairs of DNA samples from NSCLC tumor and normal lung tissue of the same patient. We observed quantitative allelic imbalance in tumor DNA in 4/19 (21%) of heterozygotes, where reduced signals of SNPs alleles could be seen compared to signals of ancestral alleles, and vice versa. This allelic imbalance was not found in any non-tumor (normal) lung DNA sample. Finally, we evaluated this allelic imbalance by quantitative Real-Time PCR of other loci in LNCR2 region by measurement of cycle threshold (CT) value of tumor DNA compared with CT value of DNA from normal lung tissue against to CT value for TFCR, a housekeeping gene.
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P06.129
A *BACH2-BCL2L1* fusion gene resulting from a t(6;20)(q15;q11.2) chromosomal translocation in the lymphoma cell line BLUE-1

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Abnormalities of the long arm of chromosome 6 are a common feature in various B-cell malignancies. In most cases the genes involved have not yet been clearly identified. We have molecularly characterized the recently established Burkitt lymphoma cell line BLUE-1 that carries a t(6;20)(q15;q11.2) rearrangement in addition to the typical t(8;14) with *MYC-IgH* fusion. To identify the gene loci involved on both chromosomes we applied a sequential BAC clone mapping strategy. Using RT-PCR we were finally able to detect a fusion mRNA transcript showing a fusion of the first (non-coding) exon of *BACH2* (basic leucine zipper transcription factor 2) on 6q15 to the second exon of *BCL2L1* (*BCL-X*) on 20q11. Various fusion transcripts were detected for different *BCL2L1 (BCL-XL)* isoforms. The fusion ultimately results in strong expression of the Bcl2l1 *(*Bcl-xL*)* anti-apoptosis protein, as demonstrated by immunoblotting. This is the first report that shows involvement of both *BCL2L1* and the transcription factor *BACH2* in a chromosomal rearrangement. It points to *BACH2* as a possibly important target in lymphomas with 6q aberrations, although other genes on 6q are probably also involved in these cases. Moreover, it suggests that other members of the *BCL2* anti-apoptosis gene family other than *BCL2* itself might also be involved in lymphoma.

P06.130
Role of EBNA-1 in carcinogenesis of line 59 transgenic mice

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Generating EBNA-1 transgenic mice (line 59, which succumbed monoclonal B cell lymphoma) was designed to explore the effects of expressing EBNA-1 independent of the remaining viral components. Transgene was generated by placing the IgH intronic enhancer (Eμ) and the Polyoma virus early promoters (Py) upstream to EBNA-1

coding sequence of EBV strain B95-8. To characterize the transgene structural status, restriction enzyme and Southern blot analysis were performed. Several aspects of the integration and expression of an EBNA-1 transgene were also explored. The 3' genomic sequence flanking the transgene were isolated by IPCR and aligned to mouse chromosome 4 band D2.3. This was achieved by Fluorescent in situ hybridization (FISH) analysis. Genomic sequences surrounding the transgene segment region was analysed by the *in silico* Ensemble and NCBI online genomic database softwares. No cellular gene was found directly at the site of integration, however, genes in the proximity of this site include Laptm5, syndcan 3, yamagushi sarcoma virus gene (Yes1) oncogene and interleukin 6 (IL-6). These genes could have oncogenic potential. The yamagushi sarcoma virus gene (Yes1) oncogene and interleukin 6 (IL-6). These genes could have potential involvement in the lymphoma process. In addition the potential cooperation of EBNA-1 with cellular oncogenes leading to lymphoma was explored by differential gene expression analysis. Genes such as small GTPase *ran*, *p53*, *Rap1GDS1* and RAG-2 were found to be differentially gene expressed.

P06.131
Integration of mutation scores in the diagnosis of Lynch syndrome reduces the need for microsatellite instability testing

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Tumor-based diagnostic techniques are currently the first diagnostic step for Lynch syndrome (LS). However, these techniques are expensive, time-consuming and have low specificity.

Our aim was to evaluate the current diagnosis of LS and to investigate the role of clinically-based mutation probability methods (MMR-pro, Wijnen) to optimize it. We included 229 consecutive patients, counseled between 2000 and 2008, who fulfilled the revised Bethesda criteria and underwent further diagnosis of LS. From them, 67 (29%) had an MSI tumor, 39 of them (17%) was due to LS. The average MMR-pro score of LS patients was 0,73 (95% CI 0,63-0,82), significantly higher than the average score of non-LS patients (p <0,001). A score of more than 0,9 was present in 12% of all cases, 55% of them were LS. An MMR-pro score <0.2 was obtained in 93 (41%) of patients, including three with LS. All three patients fulfilled the Amsterdam II (AMS II) criteria compared to only one in the non-LS group.

Based on these results a diagnostic flowchart was designed, as follows: MSI is not performed if MMR-Pro scores <0.20 (except for cases that are AMS II positive) or >0.90. In the high score group detection of the four MMR proteins and/or direct DNA-sequencing of the MMR-genes can be performed. This approach was validated in 67 additional patients: 49% could be excluded from tumor analysis without missing any LS.

Therefore, integrating mutation probability scores in diagnosis of LS, can reduce the number of tumors tested by half with the same sensitivity.

P06.132
Functional analysis of Lynch syndrome-related missense mutations in MSH6

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Inherited pathogenic mutations in the mismatch repair (MMR) genes *MLH1*, *MSH2*, and *MSH6* predispose to Lynch syndrome. Major challenges in Lynch syndrome diagnostics are the DNA variants with an unclear pathogenic nature (unclassified variants, UVs) such as single amino acid substitutions and small in-frame deletions. In particular, MSH6 UVs account for a substantial proportion of these UVs. This study has been performed to evaluate the pathogenicity of five of such inherited MSH6 UVs found in patients suspected of Lynch syndrome.

The mutated MSH6 proteins, all containing single amino acid substitutions, were tested for expression, stability and sub cellular localization in an MSH2/MSH6-proficient cell line (HEK293T). Moreover, interaction of the MSH6 mutants with MSH2 was analyzed by yeast two-hybrid experiments.

Protein expression of the MSH6 mutants p.Ser144Ile, p.Ala326Val, p.Gln522Arg, p.Ala1021Asp, and p.Thr1219Ile was comparable with the expression of wild type MSH6 co-expressed with MSH2. Analysis of *MSH6* expression levels by real time PCR corroborated these findings. These experiments showed a high similarity between the protein and the gene expression patterns. Furthermore, no effects were observed on protein-protein interactions and all five MSH6 UVs showed a normal nuclear localization. Our different functional assays yielded no evidence that the five *MSH6* UVs tested are the cause of Lynch syndrome in these patients.

P06.133
Somatic mutations in *MLH1* and *MSH2* are a frequent cause of mismatch-repair deficiency in Lynch syndrome - like tumors.

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Introduction - Lynch syndrome (previously called hereditary nonpolyposis colorectal cancer (HNPCC)) is caused by germline mutations in the mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6,* and *PMS2* and *EPCAM,* and accounts for ~3% of all colorectal cancers. Tumors associated with Lynch syndrome are characterized by microsatellite instability (MSI) and loss of expression of one or more MMR proteins. In a considerable number of patients with tumors that fulfill these criteria a germline mutation cannot be identified. To elucidate the cause of the MMR-deficiency in these tumors, we analyzed whether somatic mutations in the suspected MMR gene could explain the phenotype.

Methods - In total 13 unexplained MLH1-deficient tumors and 9 unexplained MSH2-deficient tumors were screened for somatic mutations in *MLH1* and *MSH2*, respectively.

Results - In 10 out of 22 (45%) tumors one mutation could be identified that was not present in the surrounding normal tissue. In 4 MSH2- and 3 MLH1-deficient tumors (32% in total), a second mutation or LOH was identified.

Conclusion - In 10 tumors, especially those that are MLH1-deficient, we could only identify one mutation, suggesting the presence of an as yet unidentified second mutation in either the tumor or the germline. In the 32% of cases with two tumor-specific mutations in *MLH1* or *MSH2* the presence of an additional germline MMR gene mutation is unlikely, thereby reducing their familial risk for Lynch syndrome-related tumors considerably.

P06.134
Cancer risks associated with germline mutations in *MLH1*, *MSH2* and *MSH6* genes in Lynch syndrome: results from the large nationwide French ERISCAM study

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Providing accurate and unbiased estimates of cancer risks is a major challenge in the clinical management of Lynch syndrome. This study aimed to determine the age- and gene-specific cumulative risks of developing various tumors using the largest series to date of families with *MLH1*, *MSH2* and *MSH6* gene mutations.
Enrolled from forty French family cancer clinics, 537 families with mutated genes (248 *MLH1*, 256 *MSH2*, 33 *MSH6*) were analyzed. Risks were estimated using the Genotype Restricted Likelihood method accounting for ascertainment bias (*Bonaïti et al. Eur J Hum Genet 2011; 19:173-9).*
Significant differences were found between the three mutated genes ($p < 0.01$). The cumulative risks of colorectal cancer by age 70 were 41% (95% CI: 25-70) for *MLH1* mutation carriers, 48% (30-77) for *MSH2* and 12% (8-22) for *MSH6*. For endometrial cancer, corresponding risks were 54% (20-80), 21% (8-77) and 16% (8-32) and for ovarian cancer they were 20% (1-65), 24% (3-52) and 1% (0-3). The cumulative risks by age 40 did not exceed 2% for endometrial cancer and 1% for ovarian cancer, irrespective of the gene. The lifetime risks for other tumor types did not exceed 3%.
Compared with *MLH1* or *MSH2* mutations, *MSH6* mutations were associated with markedly lower cancer risks of both colorectal and gynecologic cancers. Lifetime ovarian and endometrial cancer risks associated with *MLH1* or *MSH2* mutations were high but increased only after age 40. Our findings should lead to adaptation of preventive strategies according to the mutated gene.

P06.135
Grawitz tumour, paraganglioma, ovarian and colorectal cancer in an *MSH6* mutation carrier

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Lynch syndrome (HNPCC) is an autosomal dominant tumour syndrome caused by germline mutations in DNA mismatch repair (MMR) genes. Tumours are predominantly colorectal and endometrial, but other types have been reported, typically associated with MSH2 mutations (1). Such diversity is very uncommon in patients with MSH6 mutations. We present a 58 year-old woman with a truncating mutation in the *MSH6* gene (c.651dupT), who has been diagnosed with two recto-sigmoid adenomas (at age 26 and 30 years), ovarian carcinoma (51 yrs), a Grawitz tumour (52 yrs) and colorectal cancer (58 yrs). The colorectal and ovarian tumours were microsatellite unstable, the Grawitz tumour was not. All three tumours showed loss of the MSH6 protein demonstrated by immunohistochemical expression. Recently, our patient developed a paraganglioma; MMR staining and microsatellites will be tested after surgery.
Grawitz tumours and paragangliomas have so far not been associated with Lynch syndrome; analysis of the paraganglioma is pending. Our case demonstrates that germline MSH6 mutations can be associated with a previously unreported diversity of tumours. In general, immunohistochemistry for MMR proteins appears to be more sensitive in identifying underlying Lynch syndrome in tumours other than colorectal cancer (2).
1. Thyroid cancer in a patient with a germline MSH2 mutation. Case report and review of the Lynch syndrome expanding tumour spectrum. *R.P. Stulp, J.C. Herkert,, et.al.,* Hered Cancer Clin Pract, 2008; 6(1) 15-21
2. Differential cancer predisposition in Lynch syndrome: insights from molecular analysis of brain and urinary tract tumors. *Gylling AH, Nieminen TT, et.al.* Carcinogenesis. 2008 Jul; 29(7):1351-9.

P06.136
Seven years of MUTYH genetic screening in unrelated Czech APC-mutation-negative polyposis patients

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The MUTYH associated polyposis (MAP) is an autosomal recessive syndrome associated with 5-100 colorectal adenomas and caused by mutation in the MUTYH gene. Patients with MAP present with clinical features similar to familial adenomatous polyposis (FAP) or attenuated polyposis (AFAP) but in the absence of a strong multigenerational family history of polyposis. The MUTYH gene (NM_001048171.1) is localized on chromosome 1p.
We examined the whole MUTYH gene in 120 APC-mutation-negative probands and thereto we screened for mutations in the exon 7 and 13 of MUTYH gene in 121 APC-mutation-negative probands. Mutation screening was performed using denaturing high performance liquid chromatography (dHPLC) or high resolution melting (HRM) analysis. Samples showing unique profiles were sequenced in both directions.
We have detected 6 patients with biallelic mutation in MUTYH and 6 patients with monoallelic MUTYH mutation. The “hot spots” mutations: c.494A>G (p.Y165C) and c.1145G>A (p.G382D) were found, as well as two already reported mutations c.891+3A>C (p.Gly250TrpfsX7) and c.1105delC (p.Ala371ProfsX23).
MUTYH and MSH6 proteins act in cooperation during the DNA repair process. Based on this interaction, it was hypothesized that the combination of heterozygote germline mutations in both genes could result in an increased CRC risk. Hence we have tested the presence of MSH6 mutation in 6 carriers of monoallelic MUTYH mutation. We would like to confirm the association between MUTYH and MSH6 mutations. Unfortunately there is no evidence for this association.
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P06.137
Molecular analysis of the *TP53* and *NBN* genes in pediatric patients with medulloblastoma

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Medulloblastoma (MB) is an invasive embryonic tumor of the cerebellum and the most common malignant brain tumor in children. The main cause of MB are chromosomal aberrations. Recently, we identfied germ_line mutations in *NBN* gene as a predisposition factor for MB development. The aim of this study was to investigate the involvement of germ line mutations in both *TP53* and *NBN* genes in a group of 75 MB pediatric patients. Molecular analysis was performed by PCR-SSCP and sequencing of the whole *NBN* gene and *TP53* gene exons 5 to 8. In 6 patients the *TP53* mutations were identified, including five missense substitutions: c.481G>A in exon 5 (p.Ala161Thr), c.638A>G in exon 6 (p.Arg213Arg), c.746G>C in exon 7 (p.Arg249Ser), c.760A>G in exon 7(p.Ala254Val) , c.814G>A in exon 8 (p.Val272Met) and one insertion in exon 8 (c.801_802insC) All *TP53* gene mutations identified in this study have not been described before. No pathogenic mutation but polymorphic variants in *NBN* gene were found in our MB group: c.102G> (p.Leu34Leu), c.553G>C (p.Glu185Gln), c.1197C>T (p.Asp339Asp), c.1397+45delA, c.2016A>G (p.Pro672Pro), c.1915-7A>G, c.2120-30A>T. Our study may suggest a role of TP53 mutation in the pathogenesis of medulloblastoma.
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P06.138
Clinical features of medulloblastoma in heterozygous carriers of the *NBN* gene germline mutations

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Medulloblastoma (MB) is the most common malignant brain tumor in childhood. Epidemiological data indicate that the *NBN* gene can be considered as a susceptibility factor for cancer development. We documented significantly higher frequency of two *NBN* germ-line mutations in a cohort of Polish pediatric patients with MB (6.36%) than in general Polish population (0.5%). Aim of our study was to compare clinical features, toxicity profile and treatment outcome of MB in carriers and non-carriers of two *NBN* germline mutations, c.511A>G and c.657_661del5. Patients and methods: 98 MB patients (including 7 carriers of *NBN* mutation) were analyzed for age at diagnosis, duration of symptoms, disease stage, extent of resection, treatment complications, chemotherapy compliance and survival. Results: Median age at diagnosis was 5.17 in heterozygotes and 8.83 years in

others. There was no difference in symptoms duration. Dissemination and tumor residual were present in 43% of heterozygotes and in 29 % and 30 % of others respectively. Chemotherapy doses were reduced to 77 % and 86% respectively. Grade 3 and 4 toxicities were more frequent in heterozygotes. Event free and overall survival were better in patients without *NBN* mutation.
Conclusions: Younger age and advanced disease at diagnosis, and unwanted considerable chemotherapy dose reductions in heterozygous *NBN* mutation carriers suggest that molecular variants in this gene may have an impact on clinical course and outcome of childhood MB. To confirm our observations further investigations of larger group of patients are necessary.
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P06.139
Heterogeneity of familial medulloblastoma

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Medulloblastoma is the commonest malignant brain tumor in childhood accounting for approximately 20% of all primary brain tumors between 0 and 14 years of age. Familial medulloblastoma is rare and usually demonstrates an autosomal dominant pattern of inheritance with variable penetrance. *PTCH1* and *SUFU* are both regulators of the sonic hedgehog signalling pathway and germline inactivating mutations in both genes are associated with multisystem phenotypes including medulloblastoma. Recently, *SUFU* mutations were reported in two unrelated families with multiple cases of medulloblastoma (desmoplastic/MBEN subtype) without additional phenotypic features. We recruited three familial medulloblastoma pedigrees within the FACT study. Family 1 contains two second cousins with classical medulloblastoma, family 2 contains four affected individuals in three generations including one case of anaplastic medulloblastoma and family 3 contains two affected second cousins, one with desmoplastic medulloblastoma. We performed full-gene mutational analysis of both *PTCH1* and *SUFU* in affected individuals from each family. We identified no mutations in *PTCH1* or *SUFU* in the three familial medulloblastoma pedigrees. These data indicate that familial medulloblastoma is a genetically heterogeneous disorder with at least one further susceptibility gene to be discovered.

P06.140
Network-guided analysis of genes with altered somatic copy number and gene expression reveals pathways commonly perturbed in metastatic melanoma

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Cancer genomes frequently contain somatic copy number alterations (SCNA) that can significantly perturb the expression level of affected genes and thus disrupt pathways controlling normal growth. In melanoma, many studies have focussed on the copy number and gene expression levels of the BRAF, PTEN and MITF genes, but little has been done to identify new genes using these parameters at the genome-wide scale. Using karyotyping, SNP and CGH arrays, and RNA-seq, we have identified SCNA affecting gene expression ('SCNA-genes') in seven human metastatic melanoma cell lines. We showed that the combination of these techniques is useful to identify candidate genes potentially involved in tumorigenesis. Since few of these alterations were recurrent across our samples, we used a protein network-guided approach to determine whether any pathways were enriched in SCNA-genes in one or more samples. From this unbiased genome-wide analysis, we identified 28 significantly enriched pathway modules. Comparison with two large, independent melanoma SCNA datasets showed less than 10% overlap at the individual gene level, but network-guided analysis revealed 66% shared pathways, including all but three of the pathways identified in our data. Frequently altered pathways included WNT, cadherin signalling, angiogenesis and melanogenesis. Additionally, our results emphasize the potential of the EPHA3 and FRS2 gene products, involved in angiogenesis and migration, as possible therapeutic targets in melanoma. Our study demonstrates the utility of network-guided approaches, for both large and small datasets, to identify pathways recurrently perturbed in cancer.

P06.141
A new MEN1 mutation: report of a Belgian family

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Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant cancer syndrome that is caused by a germ-line mutation in the MEN1 gene encoding a tumor-suppressor protein, menin, on 11q13. MEN1 causes a combination of endocrine tumors such as parathyroid adenomas, pituitary adenomas, glucagonomas, gastrinomas, insulinomas, adrenocortical adenomas and non-endocrine tumors. Primary hyperparathyroidism is the most frequent expression of MEN1 with a 100% penetrance at 50y. The prevalence of entero-pancreatic islet tumors and pituitary tumors in MEN1 patients vary from 30 to 75% and from 20-65% respectively.
We here present a large MEN1 family where the carriers (n=8) developed mild hyperparathyroidism (3/8), multiple well differentiated functionally active neuro-endocrine tumors of the pancreas (2/8) and adrenal adenoma (1/8). The age range of the first symptoms was from 29y to 54y. No affected family member developed a pituitary tumor. The causal mutation is a new double substitution in the coding region of exon 2 in the MEN1 gene at position 428 where a thymidine was exchanged for an adenosine and at position 429 where a cytosine was exchanged for a thymidine (CTC>CAT), and consequently a leucine for histidine c.(428T>A; 429C>T)(p.Leu143His).
This new mutation in the MEN1 gene is clinically relevant leading to a milder spectrum of MEN1 compared to literature data. Genetic testing allowed identification of asymptomatic carriers, their early follow-up and will improve clinical outcome. Longer follow-up of carriers will allow further characterisation of this phenotype and contribute to the genotype-phenotype discussion.

P06.142
Monitoring of tumour cells redox state and real- time proliferation by novel biophysical techniques.

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Lateral prostate lobes - zinc accumulating and citrate secreting prostate parts, are the major sites of initiation of prostate cancer malignancy. Compared to healthy prostate cells, cancer cells are characteristic by decreased ability to uptake and accumulate zinc ions, which have serious impact for the cells. Zinc is in prostate implicated in important processes - energy metabolism, proliferation, differentiation and apoptosis. Therefore, it is higly expectable that zinc plays an important role in prostate cancer pathogenesis. Intracellular zinc is buffered mostly by metallothioneins (MTs), low-molecular weight cysteine-rich proteins. MTs play a key role in metabolism, transport and storage of heavy metals and protect cells against oxidative stress. In our study we focused on zinc treatment effect on PC-3 prostate cancer cell line and PNT1A, healthy prostate cells. We've been concerned on monitoring of redox status influenced by zinc ions, especially on gene expression of metallothionein 1A and 2A and then on protein and peptide level of metallothionein and reduced/oxidized glutathione. We also focused on cell quality and proliferation after zinc ions treatment. Conventionally,

most of the standard methods for cell viability or proliferation determination like MTT, XTT are based on a time-consuming end-point analysis not capable register very small and fast changes in cellular morphology. This study for the first time describes application of new, label free and noninvasive method based on impedance determination for real-time analysis of cell proliferation, adhesion and spreading as well as utilization of electrochemical methods in redox state determination concering on tumour/non-tumour prostate cell lines.

P06.143
Methylation signature of metastasis and non-metastasis lymph nodes in breast cancer patients

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Present study investigated the contribution of aberrant methylation profile of cancer related genes, *APC, BIN, BMP6, BRCA1, CST6, ESR-b, GSTP1, P14, P16, P21, PTEN,* and *TIMP3,* in paired metastasis and non-metastasis axillary lymph nodes in comparison to the primary tumor tissue and the adjacent normal tissue from the same breast cancer patients.

The quantitative methylation analysis of the candidate genes showed higher methylation proportion for the primary tumor tissue versus matched normal tissue and the differences were significant for *APC, BIN, BMP6, BRCA1, CST6, ESR-b, P16, PTEN* and *TIMP3* promoter regions (P<0.05). Among the significant methylated genes, *APC, BMP6* and *BRCA1* represented high methylation proportion in paired metastasis and non-metastasis lymph nodes compared to the normal tissue (P<0.05) whereas the *P16* promoter was methylated only in the metastasis lymph node (P<0.05). We identified even greater hypermethylation proportion of *BMP6* in the metastasis lymph node than the primary tumor tissue (P<0.05). Conversely, the promoter region of *BIN1, GSTP1* and *P14* significantly showed less methylation proportion in both the lymph node metastasis and non- metastasis compared to the matched normal tissue (P<0.05).

Taken together present study showed methylation heterogeneity between primary tumors and metastatic lesion. Contribution of aberrant methylation alterations of *APC, BMP6, BRCA1* and *P16* genes in metastasis lymph node suggests more investigation for the pathways and networks related to these genes which might improve knowledge of mechanism underlying metastasis and might improve prognosis and therapeutic strategies for the breast cancer patients.

P06.144
Methylation as a prognosis factor in patients with malignant pleural effusion

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Introduction: DNA methylation in the promoter region of tumor-suppressor genes constitutes an important mechanism in cancer development. The aim of this study was to determine if methylation analysis of *p16/INK4a*, *BRCA1*, *RARβ* and *MGMT* is a prognostic factor in patients with malignant pleural effusion (MPE).

Methods: Forty-nine patients with MPE were included. Methylation status was achieved using methylation-specific-PCR. Patients were followed-up for at least 1 year. Kaplan-Meier and Cox regression analyses were done to study survival.

Results: According to diagnosis, patients were classified as 37 lung cancers (75.5%), 5 breast cancers (10.2%) and 7 other epithelial neoplasias (14.3%). *p16*, *BRCA1*, *RARβ* and *MGMT* were found methylated in 20.4%, 57.1%, 36.7% and 30.6% patients, respectively; methylation of at least one gene was observed in 87.8% of the patients.

According to Kaplan-Meier analyses *p16* (p=0.225), *BRCA1* (p=0.796), *RARβ* (p=0.824) and *MGMT* methylation (p=0.541) were not related to survival; the absence of at least one methylated gene (p=0.048) and metastasis (p=0.048) showed association with reduced survival.

Furthermore, Cox multivariate analysis also showed that absence of at least one methylated gene (p=0.063) and metastasis (p=0.049) were the covariates that better correlated with patients' prognosis. In the subgroup of lung cancer patients, absence of at least one methylated gene (p=0.046) was more related to survival than metastasis (p=0.089).

Conclusion: The presence of methylation in at least one gene is related with a better prognosis in patients with MPE; this correlation is increased in patients with lung cancer.

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P06.145
Methylation analysis of MicroRNA-Associated CpG Islands and LINE-1 repeats in Hereditary and Sporadic Carcinomas by MS-MLPA method.

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MicroRNAs are small noncoding RNAs that contribute to tumorigenesis by acting as oncogenes or tumor suppressor genes. Many miRNA genes have associated CpG islands, suggesting epigenetic regulation of their expression. Compared to sporadic cancers, the role of miRNAs in hereditary or familial cancer is poorly understood. A custom Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) assay, developed for 11 miRNA loci, was used to study 96 CRC, 58 GC, and 41 EC, occurring as part of inherited DNA MMR deficiency (Lynch syndrome), familial CRC without MMR gene mutations, or sporadically. Compared with the respective normal tissues, the predominant alteration in tumor tissues was increased methylation for the miRNAs 1-1, 124a-1,124a-2,124a-3, 148a, 152, and 18b; decreased methylation for 200a and 208a; and no major change for 373 and let-7a-3. Interestingly, by linear correlation analysis, we found that miRNAs methylation was significantly correlated with transcriptional repression, indicating the observed methylation changes are likely to be functionally important. Moreover, hypermethylation at miRNA loci correlated with hypermethylation at classical TSG promoters in the same tumors. Furthermore, evaluation of global levels of DNA methylation using LINE-1 as a surrogate marker, were assessed by developing a second MS-MLPA assay. Preliminary observations indicate that FCCX tumors, besides including high and low methylator subgroups based on TSG and miRNA loci (studied by MS-MLPA), also showed evidence of global hypomethylation based on LINE-1. Our results highlight the importance of epigenetic events in hereditary and sporadic cancers and suggest that MS-MLPA is an excellent choice for quantitative analysis of methylation.

P06.146
Microsatellite instability detection by high-resolution melting analysis

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BACKGROUND: Microsatellite instability (MSI) is an important marker for screening for hereditary nonpolyposis colorectal cancer (Lynch syndrome) as well as a prognostic and predictive marker for sporadic colorectal cancer (CRC). The mononucleotide microsatellite marker panel is a well-established and superior alternative to the traditional Bethesda MSI analysis panel, and does not require testing for corresponding normal DNA. The most common MSI detection techniques-fluorescent capillary electrophoresis and denaturing HPLC (DHPLC)-both have advantages and drawbacks. A new high-resolution melting (HRM) analysis method enables rapid identification of heteroduplexes in amplicons by their lower thermal stability, a technique that overcomes the main shortcomings of capillary electrophoresis and DHPLC.

METHODS: We investigated the straightforward application of HRM for the detection of MSI in 70 archival CRC samples. HRM analysis for 2 MSI markers (BAT25 and BAT26) was evaluated, and 2 different HRM-enabled instruments were compared-the LightCycler® 480 (Roche Diagnostics) and the LightScanner(TM) (Idaho Technology). We also determined the analytical sensitivity and specificity of the HRM assay on both instruments using 11 known MSI-positive and 54 microsatellite-stable CRC samples.

RESULTS: All MSI-positive samples were detected on both instruments (100% analytical sensitivity). The LightScanner performed better for analytical specificity, giving a combined specificity value of 99.1% compared with 92.3% on the LightCycler 480.

CONCLUSIONS: We expanded the application of the HRM analysis method as an effective MSI detection technique for clinical samples, which can be used to include additional markers.

P06.147
Somatic and constitutional *MLH1* methylation analysis for Lynch syndrome screening and diagnosis.

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MLH1 somatic methylation in tumors has been considered as the main molecular feature of the sporadic microsatellite unstable cancers. Constitutional *MLH1* methylation, as causes of Lynch syndrome (LS), is a rare condition that might be underestimated because of an inadequate analysis strategy.

A total of 61 index subjects from Spanish families suspected of having LS and with immunohistochemical loss of expression of MLH1, were recruited from the Genetic Counseling in Cancer units from the Comunidad Valenciana. Somatic *MLH1* methylation and germline mutations were assessed by MS-MLPA and sequencing plus MLPA, respectively.

MLH1 somatic methylation was negative in 38 cases and *MLH1* germline mutations were present in 12 of them (31.6%). The remaining 26 cases were triple negative for *MLH1* expression, methylation and mutation. *MLH1* somatic methylation was detected in 23 cases (37.7%). Sixteen of these patients were analyzed for constitutional *MLH1* methylation and we found one case with *MLH1* methylation in peripheral blood, colorectal mucosa of normal appearance and oral mucosa epithelial. This subject was diagnosed of colorectal cancer at age of 30, with no familial history of cancer and initially considered as a sporadic cancer with microsatellite instability.

Our results show that 6.25% (1/16) of the tumors with loss of expression of MLH1 and somatic methylation, had actually, a constitutional silencing of *MLH1* gene by methylation. The LS genetic/epigenetic diagnosis strategy should incorporate the constitutional methylation analysis in all cases where *MLH1* tumor methylation is detected.

P06.148
Detection of splicing defects in the molecular diagnosis of the Lynch syndrome: evaluation of the effect of unclassified variants in MMR genes using minigene assays

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A fraction of the variants of unknown significance (VUS) found in genetic screenings may be deleterious by affecting RNA splicing. We have added to our molecular diagnostic procedure a functional minigene assay that assesses the impact on splicing of VUS identified in the MLH1 and MSH2 genes, involved in the Lynch syndrome. The assay relies on the use of patient genomic DNA, which is always available in the molecular diagnostic laboratory. We have so far tested a total of 167 VUS (105 in MLH1 and 62 in MSH2), corresponding to 50 intronic and 117 exonic variants, identified in the French national network of oncogenetic laboratories. We found that 49 VUS (29%) have an effect on splicing. Among them, 32 VUS (19%) lead to a major splicing defect by affecting intronic or exonic sequences close to a splice site or by creating a new splice site. In addition, 17 VUS are responsible for incomplete effects on splicing. Using an ESE (enhancer splicing regulatory element)-dependent splicing assay, we showed that 4 VUS potentially affect splicing regulatory elements. Moreover, using this assay, we identified ESE-containing sequences in eight different exonic regions of the MLH1 gene and four exonic regions of the MSH2 gene. Functional splicing minigene assays represent powerful tools to assess the impact of variants on splicing and can be used as a complementary approach to RT-PCR analyses of patient blood RNA, together with clinical and family data and tumor analyses.

P06.149
MSH6 mutation carriers and selection criteria

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The phenotype of MSH6-related Lynch Syndrome (LS) patients differs from MLH1/MSH2 patients and a Norwegian study showed that selection criteria are not sensitive enough for 3,3% of MSH6 mutation carriers. We investigated the clinical characteristics of 177 MSH6 mutation carriers from 95 families of the database of the German HNPCC-Consortium. 101 patients had a diagnosis of colorectal or LS-associated cancer between 19 and 75 years of age. 14 families fulfilled the Amsterdam-criteria, however, 15 met none of the revised Bethesda criteria, but two families would have met the original B7 criterion, which is no longer applied. Of the 16% of missed MSH6-related families, all tumours analysed indicated LS by immunohistochemical (IHC) loss of MSH6/MSH2 protein staining in all 13 tumours, and/or high microsatellite instability (MSI-H) in all 9 tumours analysed.

Considering tumours of all MSH6 mutation carriers, 64 were MSI-H, 4 MSI-L (all IHC loss of MSH6) and 5 MSS: one breast cancer, one initial MSS tumour showed MSI-H after dilution and re-testing, and in three cases another tumour in the family was MSI-H. Immunohistochemistry showed lack of MSH6 in 75 tumours -also including 4 of the 5 MSS tumours, whereas 3 tumours retained MSH6 staining but were MSI-H. We conclude, that clinical selection of potential MSH6 mutation carriers (sensitivity 84%) would be enhanced by re-inclusion of the “adenoma before age of 40 years” (original B7) and “family history with adenomas” (original B2) criterion. Furthermore, combined analysis of microsatellite instability and IHC is highly sensitive to identify MSH6 mutation carriers.

P06.150
Effects of Common Methylene Tetrahydrofolate Reductase (MTHFR) Polymorfhisms on the Risk of Bladder Cancer in Turkey.

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Bladder cancer is the fourth most common cancer in men and was estimated to be the eighth leading cause of cancer-related deaths in men in 2009. The different potential pathways for bladder cancer tumorigenesis have been shown in various studies. Many biomarkers have been used for the diagnosis, surveillance, and management of bladder cancer. Here, we used methylene tetrahydrofolate reductase (MTHFR) biomarkers. MTHFR enzyme plays a keyrole in the folate metabolism which is present in many bio-chemical pathways such as homocysteine methylation and nucleotide bio-synthesis. The purpose

of this study is to examine relationship between MTHFR gene (677 C→T and 1298 A→C polymorphisms) and bladder cancer in Turkey. For this purpose, regions where polymorphisms are located were amplified with Polymerase Chain Reaction (PCR) method. Polymorphisms were determined by using Restriction Fragment Length Polymorphism (RFLP) method with the suitable restriction enzymes which were *HinfI* and *Fnu4HI*. We found statistically significant relationship between 677 C→T polymorphism (OR=2.36; CI 95%, 1.16-4.78; p=0.015<0.05) and bladder cancer. However, we found that there was no association between 1298 A→C polymorphism (OR=0.65; CI 95%, 0.36-1.17; p= 0.151>0.05) and bladder cancer. This is the first report that found statistically significant correlation between MTHFR gene (677 C→T) polymorphism and bladder cancer in Turkey.

P06.151
MUTYH gene mutations in Polish polyposis patients

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Colorectal adenomatous polyposis, autosomal recessive MIM #608456 also called MUTYH-associated polyposis (MAP), is an autosomal recessive disorder predisposing to occurrence of colorectal cancer. MAP was described in 2002 by Al-Tassan. The number of polyps observed in MAP patients not exceeds 1000. The colonic phenotype of disease is corresponded to attenuated form of FAP. Penetrance of CRC in MAP patients is approximately 100% by age 65 years. The colorectal cancer risk in MAP patients is 93 time higher then in general population. The MAP is caused by mutations of MUTYH gene in homozygotic or compound heterozygotic state. The MUTYH gene encoding protein with glicosylase activity, involved in repair of oxidative DNA damages. The MUTYH activity avoids the chances of pairs G:C to A:T accruing as results of oxidative DNA damages. In MUTYH gene the increased frequency Y165C and G382D was observed. Those two mutations in homozygotic or biallelic state are observed in most cases of MAP with diagnosed mutation. In our study we checked prevalence of Y165C and G382D in 338 polyposis probants with out APC gene mutation. In group with one of above mentioned mutations detected, the entire coding sequence of MUTYH gene was studied by mutations screening and direct PCR product sequencing method. In our studies we detect the MUTYH mutations in 19 probants. In one probant we observed homozygote of Y165C, five probants were compound heterozygote and remaining 13 were heterozygotes.

P06.152
Survival of MUTYH-associated polyposis Patients with colorectal cancer and matched control colorectal cancer patients.

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Background: *MUTYH*-associated polyposis is a recessively inherited disorder characterized by a lifetime risk of colorectal cancer that is up to 100%. Because specific histological and molecular genetic features of *MUTYH*-associated polyposis colorectal cancers might influence tumor behavior and patient survival, we compared survival between patients with *MUTYH*-associated polyposis colorectal cancer and matched control patients with colorectal cancer from the general population.

Methods: In this retrospective multicenter cohort study from Europe, 147 patients with *MUTYH*-associated polyposis colorectal cancer were compared with 272 population-based control patients with colorectal cancer who were matched for country, age at diagnosis, year of diagnosis, stage, and subsite of colorectal cancer. Kaplan-Meier survival and Cox regression analyses were used to compare survival between patients with *MUTYH*-associated polyposis colorectal cancer and control patients with colorectal cancer. All statistical test were two-sided.

Results: Five-year survival for patients with *MUTYH*-associated polyposis colorectal cancer was 78% (95% confidence interval [CI] = 70% to 84%) and for control patients was 63% (95% CI = 56% to 69%) (log rank test *P*<.001). After adjustment for differences in age, stage, sex, subsite, country, and year of diagnosis, survival remained better for *MUTYH*-associated polyposis colorectal cancer patients than for control patients (hazard ratio of death = 0.48, 95% CI = 0.32 to 0.72).

Conclusions: In a European study cohort, we found statistically significantly better survival for patients with *MUTYH*-associated polyposis colorectal cancer than for matched control patients with colorectal cancer.

P06.153
NPM1 gene mutations in children with myelodysplastic syndromes

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Myelodysplastic syndromes (MDS) are a heterogeneous group of haematopoietic stem cell clonal disorders. MDS are rare in children and there are few studies analyzing molecular mechanisms underlying these diseases. The *NPM1* gene encodes for nucleophosmin (NPM), a protein which is, among its different functions, essential for the regulation of haematopoiesis. MDS are characterized by susceptibility to acute myeloid leukaemia (AML) and findings obtained from AML patients may serve as a clue for analyses in MDS. Mutations in exon 12 of the *NPM1* gene that cause the cytoplasmic dislocation of protein represent the most frequent genetic alterations in adult patients with karyotypically normal acute myeloid leukaemia. We used archival bone marrow samples from 17 children with MDS in order to analyze mutations of *NPM1* gene. PCR-SSCP and direct sequencing methods revealed mutation in one among 17 analyzed MDS patients. The detected mutation was the transition C to T in the codon 293 that changes serine to proline. To our knowledge, this is the first analysis of *NPM1* mutations in childhood MDS and the very first missense mutation of *NPM1* gene reported so far.

P06.154
Transcription factors are frequent targets of chromosomal aberrations in myeloproliferative neoplasms

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Myeloproliferative neoplasms (MPN) are clonal hematopoietic disorders with an increased production of terminally differentiated cells. The disease course of MPN is generally chronic, however a proportion of the patients show disease progression to secondary myelofibrosis, accelerated phase and/or secondary acute myeloid leukemia, associated with a poor prognosis. We investigated chromosomal aberrations in 408 MPN patients using high-resolution SNP microarrays to identify disease-associated somatic lesions. Of the 408 patients 37.5% had a wild type karyotype and 62.5% harbored between one and fifteen chromosomal aberrations. We identified 25 chromosomal regions that were recurrently affected (in at least three patients). In 6 of these 25 regions, the aberrations mapped single target genes including *FOXP1* (chromosome 3p), *TET2* (4q), *IKZF1* (7p), *CUX1* (7q), *ETV6* (12p) and *RUNX1* (21q). The aberrations of chromosome 4q, 7p, 7q, 12p and 21q, which overlapped with *TET2*, *IKZF1*, *CUX1*, *ETV6* and *RUNX1* respectively, were associated with disease progression, whereas aberrations of 3p (overlapping *FOXP1*) were equally distributed between disease progression stages and chronic-phase MPN. Five of these six genes (all, except *TET2*) are known transcription factors. Our data confirm the association of *TET2*, *RUNX1* and *IKZF1* with MPN, as reported before. For the first time we show that ETV6 is a target gene of chromosome 12p aberrations and

with *FOXP1* and *CUX1* we introduce two new genes involved in the disease. As the majority of the here identified genes were transcription factors, our data indicate an important, so far unrecognized, role of transcription factors in MPN pathogenesis.

P06.155
An association of three tagged SNPs of JAK2 46/1 haplotype with myeloproliferative neoplasms and non-splanchnic venous thrombosis

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Myeloproliferative neoplasms (MPNs) are hematological malignancies frequently associated with somatically acquired JAK2V617F mutation, also found in a portion of patients with splanchnic venous thrombosis (SVT). Additional, a putative association of particular JAK2 46/1 haplotype with the development of JAK2V617F-positive MPNs and SVT has been described.

288 patients with MPNs, 443 patients with non-SVT, and 458 controls were genotyped for the JAK2V617F mutation and three tagged SNPs of JAK2 46/1 haplotype.

We found that the CC+CG genotype and the C allele of rs12342421 were significantly more frequent in JAK2V617F-positive MPNs in comparison with the controls (p=5.87x10-6 and p=8.05x10-7, respectively), as well as in JAK2V617F-positive MPNs in comparison with MPNs (p=0.026 and p=0.007, respectively) and JAK2V617F-negative MPNs (p=3.83x10-4 and p=7.47x10-6, respectively). The higher prevalence of the GG+CG genotype and the G allele of rs10974944 in JAK2V617F-positive MPNs (p=9.76x10-5 and p=5.95x10-5, respectively), as well as in MPNs (p=5.87x10-4 and p<0.001, respectively) was found. In non-SVT, no significant differences in alleles or genotypes distribution of rs10974944 were observed. Furthermore, the CC+CT and the C allele of rs12343867 were significantly enriched in non-SVT in comparison with the controls (p=0.020 and p=0.013, respectively), MPNs (p=0.021 and p=1.84x10-4, respectively), and JAK2V617F-negative MPNs (p<0.001 and p=6.74x10-6, respectively).

In the present study, further evidence of the significant association of rs12342421 and rs10974944 with MPNs is provided. More importantly, the higher prevalence of rs12343867 in non-SVT indicates the related genetic background of SVT and non-SVT and reflects a feasible role of the JAK2 signaling pathway in pathogenesis of non-SVT.

P06.156
Neuroblastic tumor: Genetic profile Comparison Fresh versus cultured tumor cells

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Neuroblastic tumors (NB) are the most common extra-cranial solid neoplasm in childhood. The three histological stages of NB differ by cellular maturity and aggressiveness. In addition, NB are characterized with genetic profile high heterogeneity.

The wide variability of maturity stage and chromosomal rearrangement in tumor cell interferes closely with Tumor evolution. Certainly during cell proliferation genetic profile of tumor cells changes. It is influenced by genetic abnormality background or tumors surrounding environment. This change is reflected in tumor behavior witch fluctuates from regression to more aggressiveness.

The aim of this study is to compare genetic modifications between primitive and cultured neuroblastic tumor. Using MLPA, we followed the evolution of chromosomal rearrangement before and after in vitro proliferation of neuroblastic metastatic lymphadenopathy cells.

Our results show that in vitro proliferation leads to the loss of initial genetic abnormalities noticed in fresh metastatic tumor cells. Whereas the primitive tumor seems to be of a very bad prognosis because of its localization, size, and patients' age.

This comparative study demonstrates that analysis of genetic markers of cultured metastatic lymphadenopathy cells does not reflect the status of these same markers in fresh metastatic tissue. Such results indicate that analysis of genetic markers even performed on fresh metastatic lymphadenopathy cells does not reflect the genetic status of primitive NB. Therefore, tumor prognosis and therapeutic strategy that flows from could not rely on metastatic cells analysis.

In fact, either a differentiation or a dedifferentiation of primitive tumor cells once migrated in metastatic lymphadenopathy could occur.

P06.157
The development of the cutaneous neurofibromas

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Cutaneous neurofibromas are the hallmarks of neurofibromatosis type 1 (NF1), which is the most common cancer predisposition syndrome caused by a mutation in *NF1* gene. Cutaneous neurofibromas occur in virtually all adults with NF1 appearing at puberty and increasing in number and size with age and pregnancy. They are composed of multiple cell types, and are traditionally believed to arise from small nerve tributaries of the skin. Genetically, neurofibromas are lesions characterized by a bi-allelic inactivation of the *NF1* gene in a subpopulation of Schwann cells, but the tumorigenesis can only occur in an $NF1^{+/-}$ background. The $NF1^{-/-}$ genotype obviously provides these cells with a growth advantage, but it is not known what proportion of the Schwann cells within a cutaneous neurofibroma carries an $NF1^{-/-}$ genotype and how much of the tumor growth can be explained by the increase in the number of other cells with an $NF1^{+/-}$ genotype. In the present study, our aim was to further elucidate the pathogenesis of cutaneous neurofibromas. The histological analyses of apparently normal skin from NF1 patients revealed minute neurofibromas, presumably in the early stages of development, in the immediate vicinity of the hair follicular apparatus. Furthermore, we showed that human cutaneous neurofibromas contain multipotent *NF1+/-* precursor cells capable of differentiating *in vitro* into cell types found in neurofibromas, including Schwann cells, fibroblasts and epithelial cells. Together these results suggest that the multipotent cells present in the hair follicles may contribute to the development of cutaneous neurofibromas.

P06.158
Development of a probe-based qPCR assay to detect constitutional and tumour *NF1* deletions

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Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder caused by mutations in the *NF1* gene. Approximately 5-10% of NF1 patients bear constitutional microdeletions encompassing *NF1* and its neighboring genes. In addition, our group has recently shown that approximately 10% of dermal neurofibromas (dNFs) from NF1 patients carry somatic second hit *NF1* deletions ranging in size from ~80kb to ~8 Mb. To date, a wide range of techniques has been developed to identify copy number losses, such as Southern blot, microsatellite analysis, FISH, array-CGH and MLPA. Although quantitative PCR (qPCR) has not been frequently used for detecting DNA copy number changes, it represents an alternative methodology due to its high sensitivity and precision, relatively low screening cost and fast assay development time. We have thoroughly designed a qPCR-based copy number assay using several hydrolysis probes from the Universal Probe Library (UPL) set, specifically distributed along a ~3Mb region including the *NF1* gene. A set of DNA samples with constitutional or somatic *NF1* deletions (previously analyzed) and control samples with a diploid status of *NF1* have been used to determine the specificity and sensitivity of the assay. Our results indicate that qPCR assay

has a high sensitivity, even in contexts of mosaicism or somatic *NF1* deletion. The utilization of this technique may represent a useful quick first screening step or a validation technique for the detection of *NF1* constitutional microdeletions and also a routine method to identify *NF1* somatic mutations in dNFs.

P06.159
Metagenes associated with survival in NSCLC

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NSCLC comprises about 80% of all lung cancer cases worldwide. Surgery is most effective treatment for patients with early-stage disease. However, 30 - 55% of these patients develop recurrence within 5 years. Therefore, markers that can be used to accurately classify early-stage NSCLC patients into different prognostic groups may be helpful in selecting patients who should receive specific therapies.

Previously published dataset [1] was used to evaluate gene expression profiles of NSCLC different subtypes. Moderated two-sample t-test from R package LIMMA was used to find differentially expressed genes between all tumor samples and adjacent controls, SCC samples and AC/BC samples. The gene expression microarray results were validated using qRT-PCR . Bayesian regression analysis and Kaplan-Meier survival analysis were performed to determine metagenes associated with survival.

We identified 599 genes which were down-regulated and 402 genes which were up-regulated in NSCLC compared to the normal lung tissue and 112 genes which were up-regulated and 101 genes which were down-regulated in AC/BC compared to the SCC. Further, for stage Ib patients the metagenes potentially associated with survival were identified.

Genes that expressed differently between normal lung tissue and cancer showed enrichment in gene ontology terms which were associated with mitosis and proliferation. Bayesian regression and Kaplan-Meier analysis showed that gene-expression patterns and metagene profiles can be applied to predict the probability of different survival outcomes in NSCLC patients.

1. Vooder, T., et al., Gene Expression-Based Approaches in Differentation of Metastases and Second Primary Tumour. Case Rep Oncol.3 (2): p. 255 -261

P06.160
MicroRNA expression profiling in Estonian patients with non-small cell lung cancer

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Lung cancer is one of the most common types of cancer, showing poor survival and high relapse rate. MicroRNAs (miRNAs), small noncoding RNA molecules, are important gene regulators on transcriptional level and involved in variety of cancers, including non-small cell lung cancer. In the current study, we analyzed the expression levels of 858 miRNAs in 38 Estonian non-small cell lung cancer samples (stages I and II) and 27 adjacent non-tumorous tissue samples using Illumina miRNA array. We identified 47 up-regulated and 30 down-regulated miRNAs in cancer samples compared with the normal lung tissue ($P<0.01$). Aberrantly were expressed several well-known tumorigenesis-related miRNAs (up-regulated miR-9, miR-182 and miR-205, down-regulated miR-101, miR-130a and miR-206), as well as some miRNAs with currently unknown function (up-regulated mir-708 and miR-941, down-regulated miR-1273). We also identified one miRNA, which expression showed association with patient survival (miR-374a). The combinatorial effect of most aberrantly expressed miRNAs is predicted to affect pathways involved in cell migration, differentiation and growth, e.g. focal adhesion, cytoskeleton regulation and several signaling pathways.

P06.161
Molecular-genetic analysis of EGFR gene in NSLC patients: Searching for the Gold Standard approach?

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Non-small cell lung cancer (NSLC) is the leading cause of death related to cancer worldwide. The epidermal growth factor receptor (EGFR) is frequently overexpressed in the NSLC. Somatic mutations of the EGFR are associated with the response of advanced NSLC to EGFR-specific tyrosine kinase inhibitors. The most significant of the reported EGFR mutations were several types of deletions in exon 19 and missense mutation p.Leu858Arg in exon 21, which account for about 90% of all mutations.

In the presented study, we have analyzed EGFR mutations in NSLC tumours from 141 patients. Samples were tested by direct sequencing of exons 19 (deletions) and 20 (insertions) and by SNaPshot analysis of exons 18 (p.Gly719Ala/Ser/Cys), 20 (p.Ser768Ile, p.Thr790Met) and 21 (p.Leu858Arg, p.Leu861Gln). Altogether 9 mutated samples were identified, 6 of them carried mutation p.Leu858Arg in exon 21 and 3 harboured c.2235_2249del15 in exon 19. Mutation detection rate has been estimated at 6.4%.

To achieve an optimal quality of material and higher percentage of tumor cells, we used and compared different approaches. We have isolated tumor DNA from bronchial swabs, FFPE biopsies and also peripheral blood. However, after comparation, acquisition of adequate material for DNA analysis still remains a problem. More detailed results of EGFR mutation and variant analyses and DNA isolation approaches will be presented.

P06.162
Effects of genetic variants of CCR5 chemokin receptors on oral squamose cell carcinoma

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Purpose: To evaluate the effect of genetic variants of CCR5 chemokin receptors in the pathogenesis of OSCC

Material & Methods: 127 patients, 104 healthy individuals requiring dental treatment followed-up in the Department of Oral Diseases were included in the study. The control group had no history of cancer. CCR5 59029 and CCR5-delta 32 genes polymorphism were assessed with PCR method in the peripheral blood samples of both groups. The standard SPSS 11.0 for windows was used in all statistical tests. To determinate of the genotype and allele frequency between the groups, chi-square and Fisher's test were used.

Results: CCR5 59029 genotype and allele prevalence were higher in study group. AA genotype and A allele were higher in control group, where GG genotype and G allele frequency were higher in study group. CCR5 59029 GG genotype was found higher in the later stages of cancer (P:0,025). The tumor diameter is greater than 4 cm increased 2.18 times in patients with GG genotype (P:0,059) and 2.34 times in patients with G allele (GG + AG genotypes) (P:0,083). Keratinization presence was 3.52 times more in patients with CCR5 59029 G allele (P:0,010). Patients with AA genotypes demonstrated 3.57 times more defence against keratinization (P:0,010). There was no statistically difference in the prevalence of CCR-5 delta 32 genotype between the groups. CCR5-delta 32 II genotype was higher in patients with history of cancer in the families (P:0,029). CCR5-delta 32 D allele was higher in the later stages of cancer (P:0,010).

P06.163
Genotyping GSTT1 and GSTM1 polymorphisms in patients with oral squamous cell carcinoma

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The glutathione S-transferase (GST) genes, GSTM1 and GSTT1, play an important role in detoxification of a broad range of carcinogens contributing to development of oral squamous cell carcinomas (OSSCs). One common genetic polymorphisms of the GSTM1 and GSTT1 genes, resulting from a homozygous deletion (null genotype) leads to the lack of enzyme activity. The absence of GSTs enzymes involved in the metabolism of xenobiotics could lead to increased risk for OSSCs.

In a case-control study a possible association between GSTM1 and GSTT1 null polymorphisms and susceptibility to OSCCs was assessed by simultaneous analysis of both genes in a single reaction using real-time PCR. Genotype discrimination was determined by melting curve analysis, using DNA extracted from 84 tumor and 175 control samples. After comparison of the melting peaks of the GSTM1 (215bp), GSTT1 (480bp) and control B-globin (110bp) genes, the following results were obtained: 45% of tumor samples had the GSTM1 null genotype vs only 33% of controls samples (p=0.04; Fisher's exact test). The frequency of GSTT1 null genotypes did not show statistical difference between OSCCs and controls (p>0.05).

GSTM1 null genotype represents a risk factor for developing OSCC in Serbian population.

P06.164
Genomic profiling of ovarian cancer for chromosome 8 by array CGH analysis

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We analyzed genomic imbalances affecting chromosome 8 by array-CGH in 28 primary ovarian cancers and 9 ovarian cancer cell lines, using a home made BAC-array covering this chromosome at a mean density of 1 BAC clone/0.8 Mb. We identified regions of highly frequent gains or losses, since they affected more than 40% of ovarian cancers and determined sites showing alterations of elevated amplitude (amplifications or homozygous deletions). Doing this we also identified at least two adjacent changed clones. Finally, we characterized the smallest regions of overlap for gains. This allowed us to determine anomalies strongly associated to the disease such as: deletions at 8p23 and 8p21 or amplifications at 8q24.21 and 8q13.2 The resolution of array-CGH gives an almost direct link to the human genome sequence and, hence, to a list of candidate genes. Certain regions that we identified harbor genes that have known roles in ovarian carcinogenesis or are excellent candidates for such a role, in particular the oncogene c-myc at 8q24.12-q24.13, and the tumor suppressor gene DLC1 (8p22-p21).

Of particular note was gain of 8q13.2 occurring at a high frequency in OC, especially in serous and late stage tumors. This region has not been reported so far and it contains the zinc finger transcription factor PRD14 and the transcription coactivator for steroid receptors and nuclear receptors TIF2, which could be potential oncogenes.

P06.165
Contribution mutations of CHEK2 gene in ovarian cancer development in populations of the Volga-Ural region of Russia

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The growth of cancer pathologies are observed in Russia. 12,000 - 15,000 cases of ovarian cancer (OC) are diagnosed every year. Special attention focused on genes that are involved in DNA repair, including *CHEK2*. Germ-line mutations in the cell-cycle checkpoint kinase *CHEK2* have been associated with breast cancer. It is of interest whether *CHEK2* is also relevant to ovarian cancer pathogenesis.

In this study, we analyzed *CHEK2* mutations *CHEK2**1100delC, IVS2+1G>A, I157T, *CHEK2*(dele 9,10) in cases (n=220) and controls (n=363) from the Volga-Ural region of Russia. Protein-truncating mutation *CHEK2**1100delC was not identified neither in cases nor controls. Splicing mutation IVS2+1G>A was absent in cases but was detected with minor frequency in control samples (0,6%). Missense substitution I157T was observed in cases and controls (4,6% and 5,5%) (OR=0,82; 95% CI:0,35-1,9; p>0,05). *CHEK2*dele9,10(5kb) mutation was seen in 0,9% of woman with ovarian cancer of slavic origin without a family history OC. At one of women observed a bilateral lesion of ovarian. In addition, we investigated frequency *CHEK2**1100delC, IVS2+1G>A, I157T, *CHEK2*(dele 9,10) mutations of *CHEK2* in three populations of the Volga-Ural region of Russia (Russians (n=192), Bashkirs (n=192) and Tatars (n=153)). Missense substitution I157T was observed in 8,34% in Russians, in 1,1% in Bashkir population and in 2% in Tatars. Significant differences among populations were found between Russians and Bashkirs (p=0,01). CHEK2*1100delC mutation was observed in Tatar population (0,66%). Mutations IVS2+1G>A and *CHEK2*(dele 9,10) were absent in three populations of the Volga-Ural region of Russia.

P06.166
Germline mutations in the PALB2 gene are population specific and occur with low frequencies in familial breast cancer

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Screening of more than 7000 German breast and ovarian cancer families by PCR based techniques revealed that in high risk groups, mutation frequencies in the two known *BRCA* genes reach between 20-50%. In contrast, mutations in other predisposing genes like *CHEK2* and *ATM* were detected with very low prevalence. As still a significant proportion of our families are lacking mutations in these known genes, we decided to screen them for mutations in another recently detected predisposing gene for breast cancer, called *PALB2/FANCN.*

Using direct sequencing, dHPLC- and/or HRM-based technologies, we performed a comprehensive mutation analysis of the entire *PALB2* gene in 818 familial cases of breast cancer from Germany. All affected index patients had been screened for small nucleotide alterations in *BRCA1* and *BRCA2* and tested negative for pathogenic mutations. We found seven truncating *PALB2* mutations (six of them novel) and two novel, potentially pathogenic missense mutations. Only one mutation, previously reported in another population, was identified in the German population. Remarkably, also some cases of ovarian cancer were reported for these PALB2 families, and breast tumor samples were either hormone receptor positive or negative.

Therefore, our observations indicate firstly, low prevalence of deleterious *PALB2* mutations, supporting the existence of individually rare disease-causing mutations, and secondly, a specific mutation profile within the German population, requiring screening of each population separately. However, as PALB2-deficient tumors were shown to be sensitive to Poly(ADP-ribose) Polymerase (PARP) inhibitors, our study has implications for newly developed, favorable treatment options in familial breast cancer.

P06.167
Analysis of PALB2 gene in BRCA1/2 negative Spanish Hereditary Breast Ovarian Cancer Families with pancreatic cancer cases

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PALB2 ("Partner and Localizer of BRCA2") is a breast cancer susceptibility gene of moderate penetrance, accounting for about 1% of BRCA1/2 negative familial and early onset breast cancers. PALB2 encodes a protein that functions in genome maintenance. This protein interacts with BRCA2 and BRCA1, and it is required in the double-strand break repair. Different recent analyses suggested that the frequency of PALB2 mutations could be increased in breast cancer families with cases of pancreatic cancer.

We aimed to evaluate the contribution of PALB2 mutations in 138 BRCA1/2 negative families with pancreatic cancer cases, collected from 11 centers throughout Spain. For this, direct sequencing and MLPA analysis of PALB2 gene has been performed.

We identified two novel truncating mutations, the nonsense c.1653T>A (p.Tyr551Stop) located at exon 4 and the frameshift c.3362del (p.Gly1121ValfsX3) in exon 13 together with one missense variant in the second base of exon 3 (c.110G>A, p.Arg37His), likely affecting the splicing process. None of these families had ovarian cancer. Moreover, 11 common polymorphisms and 9 rare or unique variants of uncertain clinical significance, 7 not previously reported, have been detected.

Conclusion: In our series of breast ovarian cancer families with pancreatic cancer cases the frequency of PALB2 truncating mutations was 1.45% (2/138). Further analysis of the putative splicing variant of exon 3 as well as of the variants of uncertain clinical significance could increase this frequency.

P06.168

Detection of *BRAF* mutation status in papillary thyroid carcinoma using SNaPshot analysis

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Papillary thyroid cancer (PTC) is a common endocrine malignancy that frequently harbors the oncogenic V600E *BRAF* mutation. As a novel prognostic molecular marker, this mutation has received considerable attention in recent years for its potential utility in the risk stratification and management of PTC. Many studies have reported that the BRAF mutation is a marker of poor prognosis. The presence of the *BRAF* mutations in papillary thyroid cancer patients correlates with older age, extrathyroidal tumor invasion, distant metastases, higher tumor stage, and even higher rates of recurrent disease. Use of this unique molecular marker, in conjuction with conventional clinicopathological risk factors, to assist the prognostication of PTC is likely to improve the efficiency of contemporary management of thyroid cancer.

The aim of our study was to establish methods for rapid, sensitive and cost efficient detection of *BRAF* mutation status in tumour tissues. As we have a lot of experience with SNaPshot analysis for detection of somatic mutations, we have concentrated on this method. The sensitivity of SNaPshot analysis (2-2.5%) is comparable to commercial DxS BRAF mutation test kit (1%). We used the optimized SNaPshot analysis to detect somatic mutations in *BRAF* gene in the set of 124 patient samples, and detected mutations in 54 tumours (43,5%). SNaPshot analysis proved to be highly sensitive and cheap method, which also works with material of relatively low quality.

P06.169

Paraganglioma and pheochromocytoma upon maternal transmission of SDHD mutations.

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It is now 21 years since van der Mey and colleagues first described clear cases of hereditary, parent-of-origin related tumorigenesis. They showed that development of the rare tumor, paraganglioma, occurs exclusively upon transmission via the paternal line. The causative gene, SDHD, encodes a subunit of mitochondrial succinate dehydrogenase. The genomic mechanism behind this phenomenon was studied by Hensen and colleagues, who presented a model involving an imprinted modifier gene located on Ch11p15.5.

Here we describe three cases of apparent maternal transmission of an SDHD mutation, leading to a paraganglioma or pheochromocytoma. We established the mode of inheritance of the mutations by analyzing all available family members. We then carried out both genetic and functional analyses of the available tumors to confirm bone fide SDHD-related tumorigenesis.

We found convincing genetic and functional evidence for the maternally-related occurrence of a case of pheochromocytoma, and suggestive evidence in a pre-operative case with vagal and carotid body paragangliomas. Haplotype analysis of tumor DNA from the first patient demonstrated somatic recombination, resulting in the loss of the paternal region of chromosome 11 including the remaining functional copy of SDHD, and the maternal chromosome including the centromere and the p arm. The third case appears to be sporadic, with no functional or genetic evidence to support a causal link between the mutation and the tumor. These data conform to the Hensen model and show that the transmission of SDHD mutations via the maternal line can, in rare cases, lead to tumorigenesis.

P06.170

Comparison of MRP1 mRNA Expression between Complete Remission and Relapse Cases among Iranian Pediatric Acute Leukemia Patients

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Background and Aim: Leukemia is the most common cancer in childhood. Treatment for acute leukemia usually begins by chemotherapy and sometime continues with radiation therapy and bone marrow transplantation. Patients with acute leukemia disorder really suffer from frequent relapse after chemotherapy. Recent studies show that one of the effective causes of failure in chemotrapeutic treatment of malignant disorder such as acute leukemia is multiple drug resistance (MDR).In addition, one of the well - known gene responsible for MDR is MRP1 Gene. The aim of this study was to The aim of this study was to determines the expression level of MRP1 Gene in Pediatric Acute Leukemia patients who were in complete remission compared to relapse patients and to explore the potential for Prediction of relapse cases.

Material and Methods: In this study, using RT-PCR technique, the research team investigated MRP1 Gene expression in peripheral blood lymphocyte cells (PBMC) of forty Acute Leukemia complete remission patients and twenty relapse individuals.

Results: The results showed that the MRP1 Gene expression was significantly higher at relapse than Acute Leukemia patients who complete remission after chemotherapy.

Conclusion: In this research the association between expression level of MRP1 and response to chemotherapy was investigated present study and it was indicated that there is a MRP1 Gene over expression in Pediatric Acute Leukemia patient at relapse stage.

Keywords: Pediatric acute leukemia, MRP1 Gene, Multidrug drug resistance,

P06.171

Exome sequencing reveals candidate genes for T-cell acute lymphoblastic leukemia predisposition in children.

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Approximately 20% of pediatric T-cell acute lymphoblastic leukemia (T-ALL) patients relapse, mostly within 2.5 years after diagnosis. Detailed characterization of diagnosis and relapse samples has shown that both disease presentations are clonally related in most cases, but may also show distinct genomic features. In contrast, consecutive leukemic presentations of patients with a suspected predisposition to leukemia are expected to be fully discordant at the genomic level. Recently, we characterized diagnosis and presumed relapse samples of 22 patients with very late disease recurrence (>2.5 years), and identified eight patients with fully discordant leukemic presentations (Szczepanski et al., J.Clin.Oncol., in press). One patient showed a germline deletion comprising the recombination activating genes *RAG1* and *RAG2*, and regulatory sequences of *LMO2*, genes frequently affected somatically in T-ALL, suggesting genomic predisposition to leukemia.

In the current study, we sequenced the exomes of remission and both leukemic samples of two patients with late discordant T-ALL recurrences. In one of the patients, the two leukemic samples harbored 106 unique independent rare variants. Four genes were recurrently affected, suggesting an important role in leukemogenesis. In addition, 215 rare variants were shared between the two leukemic samples and remission sample, of which 5 nonsense, 1 canonical splice site, 4 potential frameshift and 54 non-synonymous missense variants at highly conserved positions. Filtering of these variants for known T-ALL associated genes resulted in several interesting novel candidate predisposing genes. We conclude that a considerable proportion of late T-ALL recurrences may in fact represent second leukemias, caused by predisposing germline abnormalities.

P06.172

Increased expression of Multidrug Resistance Protein 2, MRP2, in colorectal carcinomas tissues of Iranian patients

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Objective Aim of the study was to analysis the expression level of multidrug resistance related proteins, Pgp, MRP1 and MRP2 in human colorectal carcinoma.

Methods expression level of three protein was detected by immunohistochemistry method using monoclonal antibody against each MRP1, MRP2 and MDR1 protein in 50 paraffin embedded tumor and adjacent normal tissue of CRC patients. Patients (30 male and 20 female) were in stage II and III of cancer and had no received prior chemotherapy and radiotherapy.

Results 32/50, 36/24 and 24/50 of patients expressed Pgp, MRP1 and MRP2 in their tumor region respectively, while 30/50, 38/50 and 7/50 of patients expressed three protein in their normal regions. Interestingly, MRP2 was over expressed in tumor tissues of patients. MRP1 and MDR1 were expressed in most tumor and normal tissue with high intensity and extension. Also most of males expressed MRP1 protein compared to females patients (P=0.052). In the contrary MRP2 expression was observed in patients with low extension and intensity.

Conclusion Unlike MDR1 and MRP1, over expression of MRP2 was frequently observed in cancerous region compare to normal. Therefore MRP2 is intrinsically not expressed in normal tissue of CRC patients. But malignancy of tumor was leaded to over expression of it. The mechanism in which MRP2 is over expressed in malignant colorectal tumor remains to be clarified. To the best of our knowledge this is first study to evaluate three protein, MDR1, MRP1 and MRP2 in Iranian CRC patients

P06.173

Association between UGT1A1 Polymorphism and Adverse Events in Gynecological Patients Treated with Low-dose Irinotecan

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Irinotecan is indicated for the treatment of ovarian and cervical cancers in the field of gynecologic oncology. The association between UGT1A1 polymorphism and adverse events associated with irinotecan therapy has conventionally been reported in relation to the dose of irinotecan, but no consensus has been reached on this issue. It is currently thought that an association between gene polymorphism and adverse events is seen even at such low doses of irinotecan.

The dose of irinotecan used as for gynecologic cancers is lower than that used for the treatment of other types of cancer. In addition, an association with gene polymorphisms other than *28 also draws attention in east Asia. Thus, we investigated the association between these UGT1A1 gene polymorphisms and onset of adverse events in gynecological patients treated with irinotecan.

The study involved 49 patients treated with irinotecan at our hospital for gynecological cancers. UGT1A1*28, *6, *27 and *60 were analyzed. The association between each gene polymorphism and adverse events was retrospectively examined. Adverse events associated with anti-cancer drug therapy were evaluated in accordance with the CTCAE v4.0.

The incidences of grade 3 or higher neutropenia and grade 3 or higher diarrhea were significantly increased in heterozygotes for UGT1A1*28 and *6 and homozygotes for UGT1A1*6. Furthermore, the incidence of grade 3 or higher diarrhea was significantly increased in patients having any of UGT1A1 polymorphism.

These findings revealed that an association between UGT1A1 polymorphism and onset of adverse events also exists in patients treated with irinotecan at low doses.

P06.174

Molecular genetic analysis of pheochromocytoma and paraganglioma

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Pheochromocytoma is sympathetic tumour of chromaffin cells in the adrenal medulla that may produce and secrete catecholamines. This endocrine disorder occurs in approximately 75 % as a sporadic disease, or as a hereditary disorder either as a component of cancer syndromes: multiple endocrine neoplasia type 2 (germ-line mutations in the RET proto-oncogene located on 10q11.2), von Hippel-Lindau syndrome (germ-line mutations in the VHL tumour suppressor gene located on 3p26-p25) and, much lesser in neurofibromatosis type 1 (germ-line mutations in the NF1 gene) also known as von Recklinghausen disease or nonsyndromic familial disease. Germline mutations in genes SDHB (1p36.1-p35) and SDHD (11q23) that encode subunits of succinate dehydrogenase, which participate in aerobic electron transport and the Crebs tricarboxylic acid cycle, have been identified to cause susceptibility to familial pheochromocytoma.

Head and neck paraganglioma is tumour of chromaffin cells, which

arise from parasympathetic ganglia, most commonly at the bifurcation of the carotid artery (carotid body tumour). The causes of the hereditary paragangliomas are germline mutations in the SDHB, SDHC (1q23) and SDHD genes, which encode three of the four subunits of enzyme succinate dehydrogenase (SDH). Genomic imprinting might be a possible cause as mentioned in some recent studies.
Among 205 sporadic pheochromocytoma patients 7 germline mutations were found in the VHL gene. Further, 11 mutations in SDHB gene were detected. In addition, in 4 examined patients with paraganglioma we detected mutation of the start codon in SDHD gene.

P06.175
Mutagenic and Antimutagenic Activities of *Phoneix dactylifera pollen grain* Using Ames Test

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Phoneix dactylifera pollen grain is a medicinal plant that has been used traditionally to enhance energy *Phoneix dactylifera pollen grain* was screened for the potential of mutagenic and antimutagenic activity using Ames Test. For Ames Test the particularity of the strain *of salmonella typhimurium* chosen TA100 resides in the fact that undergone a specific mutation in the Histidine operon,and for this same reason it requires histidine from a foreign supply to ensure its growth.The afore mentioned strain gives rise to reverted colonies when expose to carcinogen substance (Sodium Azide)**.** Ames Test involved the pre-incubation assay against *Salmonella typhimurium* TA 100 bacterial strain in the presence and absence of metabolic activator S9 system. Grain was evaluated using two-fold value of number of revertant's colony in negative control plate as cut-off point to determine the mutagenicity effects.
The reverted mutations and the hindrance percent of *pollen grain* was 46% in Antimutagenicity test and this value in anticancer test was 49% respectively.
This is the first study that have revealed antimutagenicity and anticancer effect of *Phoneix dactylifera pollen grain*.

P06.176
Association of Polymorphisms in the Interleukin-22 and Interleukin-22 receptor alpha-1 Genes with Colorectal Cancer

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Background: IL-22 is recognized a key player in the chronic inflammatory disorders. IL-22 signals through a class II cytokine receptor composed of an IL-22-binding chain, IL-22Rα1and the IL-10Rβ subunit, which is shared with the IL-10R. Chronic inflammation is a risk factor for colorectal cancer and polymorphisms in the inflammatory genes could modulate the levels of inflammation. We have investigated 4 single nucleotide polymorphisms (SNPs) in genes of the IL-22 (rs1179251 & rs1179246) and IL-22Rα (rs4648936 & rs10794665) in Iranian population.
Methods: Genomic DNA is isolated from peripheral blood leukocytes by salting out method. Two hundred eight patients with colorectal cancer and 253 healthy control subjects were genotyped using polymerase chain reaction-restriction fragment length polymorphism. PCR-RFLP was confirmed using the sequencing.
Result: The rs1179246 G/T (3' UTR) polymorphism of the IL-22 gene was significantly associated with CRC (p=0.01). Nevertheless, the others studied polymorphisms in IL-22 and IL-22 R α did not show any significant associated with CRC risk.
Conclusion: Our data suggest that IL-22 polymorphism in 3'-UTR region (rs1179246) may contribute to CRC susceptibility.

P06.177
Polymorphisms in alcohol metabolizing enzymes in relation to develop oral and larynx cancer in patients from de Basque Country (Spain)

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Background: Alcohol consumption is a risk factor in oral carcinogenesis. Alcohol metabolism primarily imply three groups of enzymes: alcohol dehydrogenase (ADH), cytochrome P-450 oxidase (CYP) and acetaldehyde dehydrogenase (ALDH). Several studies have suggested that certain polymorphisms of those enzymes could be carcinogenic susceptibility factors associated with alcohol consumption. Due to the high incidence of oral and larynx cancer in our region, the aim of this study was to analyze the ADH1B (rs1229984), ADH1C (rs698 and rs1693482), CYP2E1 (rs2031920 and rs3813867) and ALDH2 (rs440 and rs886205) polymorphisms in our region.
Methods: We analyzed 326 samples: 46 patients with an oral carcinoma, 38 patients with a larynx carcinoma, and 242 controls. DNA was extracted from oral cytological samples using a phenol-chloroform-isoamilalcohol method. The genotypes were determined by PCR using Taqman assays.
Results: When comparing the distribution of alleles, we only found differences between the controls and HNSCC cases for ADH1B (p=0.014) and ADH1C rs1693482 (p=0.034). Significant differences in relation to the risk of developing cancer were found only in 2 polymorphisms: ADH1B rs1229984 (His: OR 0.152, 95% CI 0.04-0.584) and CYP2E1 rs3813867 (T: OR: 0.157, 95% CI 0,024-1,029)
Conclusion: The results suggest that ADH1B rs1229984 and CYP2E1 rs3813867 polymorphisms could be associated with a higher risk of oral and larynx cancer in our region.
Grant support: BIO07/CA/025 EITB-Maratoia2006, 2008111018 Health Department, and IT-192-07 Research Groups (Government of the Basque Country). X. Marichalar and A. Acha-Sagredo are supported by fellowships from The University of the Basque Country (UPV/EHU).

P06.178
Studying colorectal polyposis to identify genes that predispose for colorectal cancer

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Colorectal polyps or adenomas are the precursor lesion for colorectal cancer (CRC). The GAMBA (Genomic Analysis of Multiple Bowel Adenoma) study aims to identify CRC-predisposing genes in patients with multiple polyps (polyposis) where the involvement of high-risk mutations in the APC and MUTYH CRC-predisposing genes were excluded. Polyposis patients, particularly those diagnosed at a young age, are very likely to have a, yet unidentified, genetic predisposition to CRC.
In this study we aim to identify CRC-predisposing genes by a combined approach of homozygosity mapping and next-generation sequencing. Homozygosity mapping can identify genomic regions that harbor potential recessively inherited CRC-predisposing genes. Exome sequencing is performed on at least 50 polyposis cases with early onset, high polyp count and regions of homozygosity. The findings are validated using the second largest European cohort of familial polyposis patients (over 500 adenoma cases).

P06.179
Influence of MLH1 -93G>A promoter polymorphism in hereditary vs. sporadic colon cancer.

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The -93 SNP of MLH1 gene is associated with colorectal cancer. It has been reported to be associated with an increased risk of microsatellite unstable.
We genotyped 48 case patients and 93 control subjects from Genetic Counselling Surgery and the Hemotherapy Center of Castilla and León (Spain). DNA from peripheral blood cells was extracted and DNA extraction of tumour tissue and normal tissue from paraffin-embedded material was done by microdissection.
Tumor microsatellite instability (MSI), immunohistochemical staining

(IHC) and -93 G>A genotype were determined.
We genotyped the polymorphism -93G>A by heteroduplex analysis in capillary array (HA_CAE), three different patrons were founded: -93GG, -93G>A and -93G>A plus -269 C>G. We not founded the genotype AA in cases and controls.
The relationship between the -93 G>A polymorphism, age, sex, MSI and IHQ in familial CRC group was not statistically significant; although the odds ratio for the GA genotype in individuals with unstable tumors and a positive IHC was higher than we expect, thus GG genotype and negative IHC.
In conclusión: we found similar frequency of -93G>A in our population than in literature, the exception is that genotype AA is not present. In addition, and in contrast to the previous study we found no relationship with CRC MLH1 mutation carriers.
It not shows a correlation between GA genotype and IHC negative CRC in patients with family history.
This results shown that the -93G>A polymorphism exert a negative effect in the MLH1 gene expression and thus we found negative IHC and MSI.

P06.180
The expression profiles of genes important for prostate cancer development.

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Comparative investigation of gene expression profiles in samples of prostate cancer and distant from tumour regions was conducted. The gene expression (AR, CYP17, EGFR, HER2, m-TOR, PDGFR, PI3K, PTEN, VEGF121, VEGF165) was determined comparative to expression of GAPDH gene by RT-PCR (Step One Plus, ABI). More frequently androgen receptor (AR) gene expression was increased relatively to expression in healthy tissue: in 90% of cases more than twice and in 40% - more than 5 times. The CYP17 was activated only in 15% of cases. At least one of the angiogenic cascade genes was activated in 50% of cases but never all three genes investigated (VEGF121, VEGF165, PDGFR) simultaneously. All but one of PTEN decreasing cases (40%) were accompanied by PI3K and mTOR expression increasing. The mTOR expression was increased in 73% of cases. All activated HER2 cases (27%) coincided with increased mTOR. Likewise, EGFR was co-activated with PI3K and mTOR. It is interesting that increased PTEN expression coincided with increased mTOR but not with PI3K (increased in 50% of cases). Possibly, the mTOR may be activated in these cases by another way than through PI3K/AKT.
In summary, we shown simultaneouse activation a number of genes important for prostate cancer development. For this reason targeted monotherapy may not be effective and combined therapy aimed at several genes are required. The approach developed may be a base of diagnostics for individualized therapy.

P06.181
Association analysis in Bulgarian prostate cancer patients adds support to a newly identified susceptibility locus on chromosome 8q24

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Background: The molecular pathology of Prostate Cancer (PC) is complex with more than 30 identified susceptibility loci. Linkage, admixture mapping and GWAS have found at least three distinct regions within 8q24, associated with PC risk. Recent studies have differentiated new PC susceptibility loci on 8q24, one of them overlapping a breast cancer associated region (Al Olama AA et al, 2009; Yeagers M et al, 2009). In the current study we attempted to replicate these findings in a case-control study of Bulgarian PC patients.
Material and methods*:* Using TaqMan® method, 183 PC samples and 183 controls were genotyped. Allele, genotype and haplotype analyses were performed for the polymorphic variants rs1016343, rs4871008, rs7841060, rs620861 on locus 8q24.
Results*:* The variant rs620861 did not show association with increased PC risk. Nominally significant results were obtained for the genotypes C/T (OR=1.51, p=0.045) of rs1016343, C/T (OR=1.5, p=0.048) of rs4871008, T/G (OR=1.52, p=0.042) of rs7841060 and the risk alleles were T, C and G respectively, although not reaching significance. The association was stronger when PC cases with high Gleason score or metastasis were compared with controls. Haplotype analysis showed that the rs1016343, rs4871008 and rs7841060 are in a LD block. The haplotype T-C-G (rs1016343- rs4871008- rs7841060) was more common for patients with Gleason score above 7 (p=0.034).
Conclusions*:* The current study adds support to the new PC locus on 8q24. To evaluate the cumulative effect of risk loci on 8q24 and elucidate the mechanism of action related to aggressiveness of PC, additional studies are warranted.

P06.182
Angiotensin -I- converting enzyme (ACE) Polymorphism as genetic risk factor in benign prostatic hyperplasia and prostate cancer

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Introduction: Prostate tumors generally have a poor prognosis but the connections between tumor development and clinical outcomes are still not well understood. These connections may be determined by genetic variation. The renin-angiotensin system has been shown to play a role in prostate cancer pathology. A change in the human ACE gene occurs by an insertion (I) or a deletion (D) of a 287-bp Alu-repetitive sequence in intron 16, leading to a change in the plasma ACE level. We investigated the association of ACE gene polymorphisms and prostate cancer risk.
Materials and methods: DNA was extracted from 66 samples from individuals with a prostate cancer diagnosis and 80 samples from individuals with a benign prostatic hyperplasia diagnosis. The ACE I/D genotypes were determined by PCR- RFLP analysis.
Results& Conclusion: The comparative analysis of the two groups revealed no significant differences for any of the ACE genotypes. No association between variant genotypes and risk of developing prostate cancer was observed with the ACE variant genotype , but it looks that further samples is needed to produce more significant results.

P06.183
Gene expression of Periostin and SHB in prostate cancer

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SHB (adapter protein) is involved in receptor tyrosine kinase signaling and apoptosis regulation. Periostin is a 93 kDa N-glycoprotein, involved in cell adhesion and extracellular matrix structure. Hepsin is a transmembrane serine protease. A detailed analysis of expression of all three genes together has not been conducted in prostate cancer so far.
We evaluated periostin, hepsin and SHB expression in prostate cancer samples by semiquantitative RT-PCR in two cohorts, including a control cohort (n=21, benign hyperplasia of prostate) and tumor cohort (n=55). We analyzed 2 samples classified as T1, 33 samples as T2, 17 as T3 and 3 samples with T4 TNM classification. Relative gene expression of the SHB was significantly higher in control samples (μ = 0.451±0.290) than in tumor samples (μ = 0.351±0.184; p<0.041). Relative gene expression of the periostin gene was higher at control samples (μ = 0.421±0.290) than at tumor samples (μ = 0.351±0.184). Relative gene expression of the hepsin gene was higher at tumor samples (μ = 0.075±0.061) than at control samples (μ = 0.016±0.013).

No correlation of the gene expression with the TNM classification, Gleason score, PSA level and age except the T(1+2) versus T(3+4) expression by hepsin was observed.
It is concluded that the SHB overexpression may be related to decreased tumor growth in prostate cancer and conversely hepsin up-regulation may be related to increased tumor aggressiveness. These data are significant for therapeutic intervention in both early and metastatic prostate cancer.
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P06.184
Aberrant promoter methylation of GSTP1, APC, CycD2 and RASSF1A as potential biomarkers of prostate cancer diagnosis and prognosis

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Substantial evidence supports the view that epigenetic changes have a role in prostate cancer (PC) development and prognosis. Analyses of the patterns of promoter hypermethylation in different genes have identified specific alterations that may serve as useful diagnostic and prognostic biomarkers. In order to identify and characterize methylation markers in PC patients from Macedonia, we used QMSP to evaluate a panel of 4 genes among 55 samples of benign (BPH) and malignant (PC) prostate lesions. The frequencies of methylation of all 4 genes were significantly higher in PCs (GSTP1 57.14%, APC 82.86%, CycD2 54.29% and RASSF1A 80%) compared to BPH (GSTP 1 5%, APC 10%, CycD2 5% and RASSF1A 10%; P<0.001), which was not age related. The methylation index (MI), defined as ratio of methylated genes to the total number of analyzed genes for each case, was significantly different in malignant compared to nonmalignant tissue (P<0.0001). The combined analysis of selected genes allowed for distinction of PC from BPH with sensitivity of 88.6% and specificity of 90.0% (AUC=0.898). Furthermore, the methylation frequency of GSTP1 and the overall MI were higher in PCs with high Gleason scores (≥7) than in those with low GS (≤6) (p=0.012 and p=0.015) respectively, suggesting association with clinicopathological features of poor prognosis. No statistically significant correlations between MI and age or preoperative PSA levels were found. Our data suggest that promoter hypermethylation is a frequent event, occurs early, and accumulates during prostatic carcinogenesis. High MI might serve as a potential biological marker for aggressive PC.

P06.185
The Prevalence mutation of PTEN gene(7,8 exones) in colorectal carsinoma(CRC)in Iranian patient`s

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Colorectal cancer(CRC) is the third most common cancer, with a multiple process with tumours presenting various genetic and epigenetic alterations inactivating tumour suppressor genes and/or activating oncogene. PTEN/MMAC1/TEP1 is a tumour suppressor gene encoding a dual- specificity protein phospatase with homology to the cytoskeleton protein, chiken tenssin and bovin auxilin. PTEN acts by inhibiting the activation of Akt/Protein kinase B and is therefore involved in a major pathway controlling cell proliferation PTEN protein has been found to be important for its activity as a tumour suppressor. Alterations have been found to be common in sporadic colorectal tumours. Recently, mutation at an $(A)_6$ repeat of PTEN exons 7 and 8 in colorectal cancer(CRC) patients with microsatellite instability have been detected. Our subjective was to identify PTEN mutation in colorectal tumours and tissue samples from Iranian patients. The entire coding region and flanking sites of the 7,8 exones of PTEN gene was amplified and conformation sensitive gel electrophoresis(CSGE) and sequencing was done to identify alterations. We analysed genomic DNA from 30 tumour tissue samples. We found no PTEN alterations among the colorectal cancer samples we had examind and thereby conclude that there is a lack of PTEN involvement in the carcinogenesis of colorectal cancer in these patients. So we recommend to investigate that level of expression of PTEN gene, such as transcriptom and protein or hyper metilation of PTEN.
Keywords: PTEN, Mutation, Colorectal carcinoma, CSGE

P06.186
Mutation screening of RAD51C in Breast and Ovarian Cancer families from Castilla-León (Spain).

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BRCA1 and BRCA2 are the two major genes involved in hereditary breast and ovarian cancer (HBOC). However, mutations in these genes explained only 20% of the familial cases, so the current interest is search for alterations in other susceptibility genes and to identify variants by genome-wide association studies that confer small increases in risk. Meindl and co-workers provided evidence that monoallelic RAD51C mutations were responsible for only 1.3% of BOC German families, but the penetrance could be similar to BRCA's. Screening in Canadian and Chicago population has been carried out without positive results. Therefore, we selected high-risk BOC families BRCA-negative to assess the incidence of RAD51C mutations in our region.
We received 89 families that fulfilled familial history of BOC, 32 of which were carriers of a pathological mutation in BRCA1/BRCA2 genes. Afterwards, we selected 27 families with three or more combined breast and ovarian cases to perform RAD51C genetic testing. The whole coding sequence and intron-exon boundaries were scanned by direct sequencing. Three previously reported variants (c.26C>T, c.904+34T>C and c.859A>G) and a novel variant in three different families (c.838-37G>T) were identified, although Splice-site prediction programmes indicated this novel variant as benign.
The proportion of BOC families available in our population is only 8.5%, a third of which are BRCA1/BRCA2 mutation carriers. Preliminary data suggested a very low prevalence of RAD51C mutations, so its screening would not be recommended in the HBOC-prevention strategy, although analysis of more families would be required to confirm this point.

P06.187
RAD51C mutations in familial breast and ovarian cancer

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Germline mutations in a number of genes encoding components of the homologous recombinational repair (HRR) pathway confer increased susceptibility to female malignancies. Recently mutations were identified in *RAD51C*, a key player in HRR, in families with both breast and ovarian cancer. It is suggested that *RAD51C* is a high penetrance cancer susceptibility gene comparable to *BRCA1* and *BRCA2*, although formal evaluation of the risks associated with *RAD51C* mutations has yet to be undertaken. Follow-up studies by other groups have failed to identify *RAD51C* mutations in additional series.
We screened *RAD51C* by direct sequencing in 618 families with both breast and ovarian cancer and 622 controls. We identified four deleterious mutations in cases and none in 622 controls (P=0.06). The mutations were not equally distributed within the series; three mutations were detected in the 166 families with two or more cases of ovarian cancer (P=0.009). Testing for the family mutation in samples from 9 relatives revealed that one of two females affected with breast or ovarian cancer had the mutation, whereas six of seven unaffected female relatives did not carry the family mutation. We are currently undertaking segregation analysis to refine the risks of breast and ovarian cancer associated with mutations in *RAD51C* and this data will be presented. These data confirm the original report of *RAD51C* mutations in breast-ovarian cancer families but suggest that the contribution of *RAD51C* mutations to familial breast-ovarian cancer is very small.

P06.188
RB1 Gene Mutations in Iranian Patients with Retinoblastoma: Report of four novel mutations

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Mutations in RB1 gene lead to Retinoblastoma which is the most common intraocular tumor in the children under the age of six. In present survey, the mutations of eighteen unrelated Iranian retinoblastoma patients were characterized for the first time.

Mutation analysis of RB1 gene was performed in patients by sequencing of all coding regions and MLPA analysis. Clinical signs and symptoms of the retinoblastoma patients appeared to be similar to previously described patients with retinoblastoma.

Eight known mutations and four novel mutations in the patients (c.832_833insT, c.1943delC, c.1206C>T and c.2029delG) were determined. Structural analysis of the c.1206C>T variant showed that exon 12 contains an SC-35 consensus sequence and this variation disrupt the splicing enhancer element and causes skipping of exon12. Molecular genetic testing of retinoblastoma patients has great impact on genetic counseling of the families involved, and management of the disease in the patients and at risk relatives.

P06.189
The microRNA profile of ccRCC-derived cell lines

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Clear cell renal cell carcinoma (ccRCC) is the most prevalent subtype of kidney cancer. ccRCC is thought to originate from the proximal epithelial tubular cells (PTEC) of the kidney. It is now well established that noncoding RNAs, most notably the class of microRNAs, can play a important role in cancer development and progression. We assessed the miRNA expression profile of a panel of 10 clear cell renal cell carcinoma derived cell lines and compared this with the miRNA expression profile of two PTECs. Eight microRNAs were significantly more abundant (p=0.05) in the ccRCC cell lines as compared to the PTEC cells. Fifteen microRNAs were significantly less abundant in the ccRCC cell lines. The latter set includes all members of the miR-200 family and miR-205 that are known to be involved in the epithelial- to mesenchymal transition process by directly repressing transcription factors ZEB1 and ZEB2. These transcription factors on their turn strongly repress the expression of epithelial marker E-cadherin, what indeed is observed in nine out of ten of our ccRCC-derived cell lines. As microRNAs bring about a posttranscriptional downregulation of their target genes, we compared the miRNA profiles with the gene expression profiles of this set of ccRCC derived cell lines. The set of significantly upregulated genes appeared to be 4-fold enriched for genes that were validated targets of the miRNAs significantly downregulated in our panel of ccRCC cell lines. The expression levels of several genes showed an anticorrelation with the respective miR levels.

P06.190
The gene expression profiles by RT-PCR in renal cancer tumor.

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The expression of genes, mainly targets for renal cancer therapy (VEGFR1, VEGFR2, VEGF121, PDGFRα, PDGFRβ, PI3K, PTEN, AXL, mTOR, EGFR) was investigated by RT-PCR. The most commonly elevated expression in renal cancer relatively to expression in healthy renal tissue (distant from tumour regions) was observed in the genes of VEGFR1, VEGFR2 and VEGF. Increased expression of at least two of these genes was observed in most of cases, half of the cases have been activated all three of these gene. A frequent lowering PDGFRα expression was found. The level of expression is most pronounced (one to two orders of magnitude) differed in tumors relative to normal tissue in the genes of VEGFR1 and PDGFRα. Moreover, the lowest level of gene expression PDGFRα (500 times below normal) corresponded to the smallest change in gene expression VEGFR1 in tumor compared with healthy tissue. Conversely, the unchanged level of gene expression PDGFRα correspond to an increase of approximately 80 times the level of VEGFR1 gene expression in the tumor. In half of the cases expression of the EGFR was increased. The PTEN expression was observed as increased 4-fold in tumor and so reduced by 3 times compared to control tissue.

The findings provide information about the gene functional features in renal cancer and can be used in future for the development of optimal schemes of effective individualized treatment of patients with renal cell carcinoma.

P06.191
Investigation of Chromosomal Abnormalities on Sporadic Renal Cell Cancer Cells

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Renal cell carcinoma (RCC) represents 2- 4% of all human neoplasm. Clear cell renal cell carcinoma (CRCC) is the most common type of renal cell carcinoma, and papillary subtype (PRCC). CRCC are often characterized cytogenetically by structural chromosome aberration of different chromosome an especially short arm of chromosome 3, and PRCC are characterized by trisomy 7 or 17 and loss of the Y chromosome. FISH is a robust and highly accurate method for detecting losses, gains and translocations of genetic material . Numeric change of chromosomes 3, 7, 9, 17, and deletion of 9p12 region were analysed in 55 patients with renal cell carcinomas and normal tissue by using fluorescent in situ hybridization (FISH) technique. We report the incidence of chromosomes 3, 7, 9, 17 aneuploidies, and chromosome 9p12 region status and also correlate with clinical parameters.

P06.192
The analysis of somatic mutations of the VHL gene in clear cell renal carcinoma patients from Bashkortostan Republic of Russia

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Kidney cancer is a heterogeneous group of malignant tumors, the vast majority of which are renal cell carcinomas of various morphological types. One of clear cell renal carcinoma (CC-RCC) features is the inactivation of tumour suppressor gene *VHL* (von Hippel-Lindau).

The aim of investigation was to analyze somatic mutations in *VHL* gene in 72 CC-RCC patients from Bashkortostan Republic. SSCP analysis followed by direct DNA sequencing was used to investigate somatic *VHL* alterations.

Mutations of *VHL* have been identified in 10 of 72 samples (13.9%) with CC-RCC. All the identified mutations were somatic and found only in tumor tissues that allowed us to exclude a hereditary von Hippel-Lindau syndrome and consider all cases as sporadic forms.

Eight types of mutations were detected in 10 CC-RCC patients: 2 insertions and 8 point mutations in the heterozygous state. Insertions resulted in frameshifts. In the 2nd exon of the *VHL* gene we revealed: p.His115Tyr (in 1.4% of patients), p.Asp143Glu (1.4%) and p.Pro154Leu (2.8%), which change the structure of the binding of *HIF-1α* in β-domain. In the 3d exon of the *VHL* gene we revealed mutations: p.Val170Phe at 1.4% of patients, p.Arg176Trp (1.4%), p.179_Leu178dup (1.4%), p.Glu186_Asp187ins (2.8%), p.Glu186Lys (1.4%), which involve a violation of section interaction elongin C α-domain. We also revealed somatic mutations p.Trp117Arg (1.4%), p.Leu128Val (1.4%) and p.His110Pro (1.4%) that haven't been described previously. Mutations of *VHL* gene result in stabilization of hypoxia-inducible factors, and may contribute to cancer progressions, that allow to consider *VHL* gene as a prognostic marker of RCC.

P06.193

A study of polymorphic xenobiotic-metabolizing enzyme genes in sporadic renal cell carcinoma patients from Bashkortostan Republic (BR) of Russia

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Renal cell carcinoma (RCC) accounts for 80-85% of malignant renal tumors in adults. The pathogenesis of RCC may be related to higher levels of exposure to certain risk factors. Epidemiological studies showed that environmental factors may be involved in sporadic RCC development.

This case-control study was designed to test for an association between genetic polymorphism of xenobiotic metabolism enzymes and risk of sporadic RCC. Genomic DNA was obtained from 130 patients with RCC (Russians, Tatars, Bashkirs) and 117 controls matched for age, gender, ethnic origin and area of residence. We used PCR-RFLP to investigate polymorphism for the most common alleles at GSTM1 and CYP1A1.The null genotype frequency distribution of GSTM1 gene (0/0 GSTM1) in RCC patients didn't differ statistically significanty from that of the control group (P>0.05). There were interethnic distinctions between control groups of Turkic and Slavic origins. The frequency of 0/0 GSTM1 genotype in Turkic group was 0,291, whereas in Slavic -0,444 (χ2=5,133, P=0,023). The analysis of Ile462Val polymorphism between patients and controls didn't reveal statistically significant differences (P>0.05). There was a higher risk of RCC for Turkic group of patients with *Ile/*Ile of CYP1A1 combined with null GSTM1 genotype (χ2=3,933, P=0,047, OR=2,276, 95%CI=1,002-5,169). The combination of non-null GSTM1 and *Ile/*Val CYP1A1 genotypes in Turkic group of patients also was a risk marker for RCC development (OR=10,705, 95%CI= 0,562-203,907). These findings suggest that variation in the metabolic pathways involved in the functionalization and detoxification of specific xenobiotics is an important susceptibility factor for RCC in Bashkortostan Republic.

P06.194

Karyotypic characterisation of childhood renal tumors

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The most common renal neoplasm in children is Wilms´ tumor (WT). Successful treatment of relapsing or nonresponding WT remains a challenge. Cytogenetic abnormalities of presumed prognostic significance include karyotype complexity and allelic losses from the short arm of chromosome 1 and from the long arms of chromosomes 11, 16 and 22 and 1q gain. Genetic alterations seem to be of importance for morphology and tumor growth in distinct subtypes of renal cell carcinomas (RCC), thus distinct abnormalities may assist histologic subtyping. Reports of chromosomal analysis of RCC in children are scarce.

We present a series of 27 WT, mostly without pre-operation chemotherapy, 2 congenital mesoblastic nephromas (CMN) and 3 rare childhood renal tumors - RCC. We have detected numerical as well as structural changes in WT, which mostly involved chromosome 1, either presenting with a deletion of 1p or the formation of an isochromosome 1q. One WT presented with an i(7)(q10) as the sole cytogenetic change, an aberration characteristic for adult WT. One RCC patient displayed the t(X;1) as the sole cytogenetic abnormality, another one showed numerical aberrations similar to those seen in adults. To gain further insight into genetic events responsible for the pathogenesis, arrayCGH was performed. This technique yielded results not seen by cytogenetics or in cytogenetically unsuccessful cases. Thus we have found non-random losses of 16q12.1-16q24.3 and 17p. In our report, we have correlated these findings with histological and clinical features, patient outcome and the results of previously published studies. **Supported by grant VZ MZ 6715.**

P06.195

The effect of Caffeic acid Phenethyl ester and Resveratrol on the down-regulation of the in vitro growth of human breast cancer cells

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Several compounds are used in traditional and alternative medicine. Caffeic acid Phenethyl ester (CAPE) is a component of beehive propolis and because of its anti-inflammatory, antiviral and anticancer properties, it has been used for treatment of several diseases including the breast cancer. Resveratrol, a compound found primarily in red grapes, has been reported to inhibit cell growth and induce apoptosis in various solid and hematological cancer cell lines.

The purpose of our study is to determine the expression profiles of 48 cell cycle control genes following the treatment of induced MCF-7 cell lines with the IC50 doses of resveratrol, CAPE and resveratrol-CAPE combination.

The IC50 values of resveratrol and CAPE for MCF-7 cells were found as 150µM and 75µM respectively, by XTT assay. Total RNA was isolated from the cells exposed to IC50 doses of resveratrol, CAPE and resveratrol-CAPE combination, the expressions of 45 genes each from apoptotic pathway and cell cycle controls were studied by real time online RT-PCR. Results were compared with resveratrol or CAPE -free cells.

The expression profiles of CCND2, RB1, ATM, CDC34, CDK5RAP1 genes showed a significant increase, compared to the control cells. Up-regulation of these genes following the treatment with resveratrol and CAPE, provide evidence that these compounds may serve as potentially effective chemoprevention agents.

P06.196

A new germline mutation at codon 918 of the RET protooncogene in a patient with concurrent lymph node metastases of medullary and papillary thyroid carcinoma

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We report a new germline RET mutation (c.2752 A>G; p.M918V) found in a 62-year-old man with synchronous medullary thyroid carcinoma and papillary thyroid carcinoma in cervical lymph node metastases. In 2006 patient underwent total thyroidectomy based on the cytological diagnosis of PTC. Postoperative histopathologic examination of the thyroid showed multifocal bilateral PTC (T3N0M0). In 2010 the patient presented with recurrent disease and underwent surgical treatment including resection of neck lymph nodes. Histological and immunohistochemical studies revealed the presence of concurrent MTC and PTC metastases in all resected nodes, with significant predominance of MTC. Postoperative CT was 20 pg/ml (N=2-6 pg/ml). Pheochromocytoma, hyperparathyroidism and MEN 2B associated abnormalities were excluded. Familial history, clinical and biochemical examination of first-degree relatives were negative for MEN 2A and familial medullary thyroid carcinoma. Genetic screening showed that one of them was a carrier of new ATG>GTG transition at position c.2752 of the RET protooncogene (serum CT - 5.9 pg/ml). A 96-year-old mother of the patient has not this mutation. The father was not available because he died of a stomach malignancy while 66. The mutation was not revealed in 50 unrelated normal individuals.

Conclusion: we detected a new germline mutation within the catalytic core of the RET thyrosinkinase domain converting methionine 918 into valine in proband and his healthy sun (37-year-old). Apparently this mutation is associated with late manifestation of MTC in the proband. But transfection studies with RET (M918V) mutant are needed to understand whether the discovered transition has oncogenic potential.

P06.197

Abnormal splicing pattern caused by a novel samesense mutation in an Iranian retinoblastoma patient

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Mutations in RB1 gene lead to Retinoblastoma which is the most common intraocular tumor in the children under age of six. Retinoblastoma is a malignant proliferation of immature retinal cells. To date a wide spectrum of the mutations have been reported in RB1 gene; in other hand, mutation analysis has great impact in the management of the disease in the affected families. Many of the reported mutations in the human genes, affect splicing; some mutations target the main splicing sequences and some altered splicing regulatory elements.

In the present survey, mutation analysis was done in an Iranian patient with sporadic unilateral retinoblastoma using direct sequencing and MLPA. As a result, a same sense nucleotide change (g.70320C>T) was found near the 5' end of exon 12. This alteration, disrupt the consensus sequence of an exonic splicing enhancer and changes the binding site of SC-35 protein. Structural analysis of cDNA in this patient showed disruption of normal splicing pattern and skipping of exon 12 from the RB1 transcript. Based on our findings it is reasonable to mentioned the above nucleotide change as a pathogenic mutation. Also for the first time we report evidence for presence an exonic splicing enhancer in exon 12 of the RB1 gene.

P06.198

Parent-of-origin effect in SDHD hereditary tumours explained by requirement for additional molecular steps.

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In SDHD mutation families, paragangliomas and phaeochromocytomas usually occur only after paternal transmission of the mutation. This unexplained parent-of-origin effect is not due to imprinting of SDHD itself as SDHD is biallelically expressed in several tissues. In paternally inherited SDHD there is loss of the entire maternal chromosome 11 in tumour DNA, implying that tumourigenesis requires loss of maternal SDHD and a further, imprinted, tumour suppressor gene (TSG).

We report the second case of an SDHD-related tumour (a phaeochromocytoma in a 33 year old woman possessing the common pathogenic mutation, p.Pro81Leu) occurring after maternal transmission. It is the first reported investigation of tumour DNA in this situation. Tumour DNA revealed loss of heterozygosity (LOH) at paternal 11q23 causing loss of the wild-type SDHD allele and LOH affecting maternal 11p15, including H19. These two LOH regions were separated by a region exhibiting clearly retained heterozygosity, containing SDHAF2 (a recently reported paraganglioma TSG), which therefore appears uninvolved here.

This case provides molecular evidence that the tumourigenic requirement for maternal 11p15 loss (in addition to inactivation of both SDHD alleles) drives the observed parent-of-origin effect. Paternal inheritance of SDHD mutations is more commonly associated with tumour formation as the necessary loss of both the wild type SDHD allele and maternal 11p15 can occur by a single event (loss of maternal chromosome 11). These findings have important implications regarding the clinical management of carriers of maternally inherited SDHD mutations - who we confirm can develop phaeochromocytomas - and the understanding of the parent-of-origin effect.

P06.199

Selenoprotein gene variants and selenium (Se) plasma concentration are major risk factors for breast and prostate cancers

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Aim

Evaluation of associations between selected selenoprotein genomic variants, total Se plasma concentration and breast and prostate cancer risk.

Material and methods

Patients

105 pairs of consecutive patients (unselected except for exclusion of BRCA1 carriers) and unaffected females matched for year of birth, adnexectomy, cancer family history and smoking were included for breast cancer studies. 81 pairs of unselected patients and unaffected men matched similarly were included for prostate cancer studies.

Genes

The most polymorphic four SNPs were identified among selenoprotein genes (GPX1, GPX4, TXNRD2, Sep15) by sequencing of representative cases.

Se plasma level was analyzed using spectroscopy (Analyst600).

Statistics

Depending on Se level quartiles including at least 25 persons each were identified for all possible genotypes.

Results

The strongest associations have been detected for genotypes listed in table below.

Cancer site	Genotype	Quartiles compared	Se concentration range (µg/l)	Cases/controls in compared groups	Fisher's Exact Test		
					p	OR	CI
Breast	TXNRD2 (rs 1139793) GG	II/III vs I/IV	59.6-79.2 vs 40.2-59.0 and 79.3-106.4	21/35 vs 38/17	0.0012*	0.27	0.12-0.59
	GPX1 (rs 1050450) CC	I/II vs III/IV	40.2-69.8 vs 69.9-107.9	32/18 vs 22/29	0.0465	2.34	1.052-5.22
Prostate	GPX4 (rs713041) nCC	I/II vs III/IV	22.0-71.4 vs 71.6-136.8	33/17 vs 20/31	0.0096*	3.01	1.34-6.77

* significant after Bonferoni correction

Conclusion

Option of diet aimed to keep serum Se level associated with the lowest cancer risk may be considered by persons with increased risk of breast/prostate cancers provided their genotype is determined.

P06.200

Molecular and clinical findings in Czech juvenile polyposis families

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Juvenile polyposis (JP) is an autosomal dominant syndrome characterized by the occurrence of juvenile polyps and predisposition to cancer of the gastrointestinal tract (GIT). Characteristic feature of juvenile polyps are irregular cystic glands filled with mucus not observed in other colorectal cancer syndromes. Germline mutations in the *SMAD4* and *BMPR1A* genes are found in 40% of the JP individuals. Hereditary hemorrhagic telangiectasia (HHT) and higher frequency of gastric polyposis are associated mostly with *SMAD4* mutations. We report here on clinical findings and molecular analysis of 12 individuals from 4 Czech families. Two probands were initially analysed for the suspicion of familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) syndromes. We analysed entire coding regions including the splice-site boundaries of the *SMAD4* and *BMPR1A* genes in genomic DNA of the probands by sequencing analysis and multiplex ligation probe-dependent amplification (MLPA) assay. By direct sequencing of the genes we have identified three pathogenic mutations (p.Gln442X, p.Pro198GlnfsX4, p.Arg445X) in *SMAD4* gene only. Seven germline mutation carriers from three families had various manifestation of the disease. No mutation was found in a sporadic patient with cutaneous telangiectasias and GIT juvenile polyps. In summary, due to variable penetrance and expressivity of the disease it is extremely important to know family history and information related to the number, localization, size and histology of the polyps predominantly to establish a correct diagnosis and further follow-up.

P06.201
Low mutation rate in the mutation cluster region of the *APC* gene in patients with sporadic colorectal cancer in Southwest Iran

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Introduction: Colorectal cancer (CRC) is the third most common cancer in men and the second in women worldwide. The majority of CRC cases are sporadic that occur in persons without a family history of CRC and are caused by somatic mutations. Adenomatous Polyposis Coli (*APC*) is a tumor suppressor gene of Wnt pathway and is mutated in 34-80% of sporadic CRC cases. About 60% of all mutations in *APC* occur in the mutation cluster region (MCR) between codons 1286 and 1513 in exon 15. Our objective was to identify mutations in *APC* gene of sporadic CRC patients.
Methods: Tumor and normal tissue samples were obtained from 29 Iranian sporadic CRC patients. We designed four primer pairs due to amplifying four overlapping fragments (codons 654-885, 853-1242, 1213-1482 and 1404-1613) of exon 15. Direct sequencing was used for analyzing of four amplified fragments.
Results: We found four frameshift mutations c.2804dupA, c.4317delT, c.4464_4471del and c.4468_4469dupCA, three missense mutations T1079S, P1176L and E1317Q and two nonsense mutations Q1367X and R1450X in eight patients. One of patients showed two mutations.
Conclusion: Mutations were detected in 8 (27.6%) of 29 CRC patients. This mutation frequency is lower in comparison to the previous studies from other countries. The c.4468_4469dupCA and T1079S are novel mutations. The identified six frameshift and nonsense mutations are pathogenic and lead to truncated APC protein. The pathogenicity of the T1079S and P1176L missense mutations is unknown but the E1317Q mutation is considered being a predisposing variant for the CRC progression.

P06.202
Case-control *association* study of COX-2 gene polymorphism (-765G>C) with Sporadic colorectal cancer (CRC)

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Background : Colorectal cancer (CRC) is a common disease in both men and women. CRC is ranked the third most common form of cancer worldwide in terms of incidence .Cyclooxygenase (COX) is a key enzyme in the formation of prostaglandins, and an inducible isoform of COX, COX-2, has been implicated in colorectal carcinogenesis.
Aim: The aim of our study was to investigate associations of COX-2 polymorphism (-765G>C) with risk of colorectal cancer (CRC) in an Iranian population.
Methods: The study subjects were 115 cases of colorectal cancer and 62 controls with no polyps who underwent total colonoscopy. Genotypes were determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method with genomic DNA extracted from the buffy coat.
Result: Our finding revealed that a significant difference between cases and controls (P=0.001).Therefore -765G>C polymorphism was associated with colorectal cancer development .Moreover, GG genotype was detected just in cases.
Conclusion: These findings suggest a modulating role for the COX-2 polymorphism (-765G→C) in the development of colorectal cancer in an Iranian population.

P06.203
STAT3 a Molecular Target in High-Grade Gliomas.

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Background: High-grade gliomas are the most common primary brain neoplasms and are generally lethal. Disruption of EGFR was defined as critical for gliomagenesis and anti-EGFR therapies were believed to be an attractive therapeutic approach to the classical regimens, however, they revealed no improvements.
Aim: The goal of this work was to analyse the expression of genes that make-up the EGFR activated signalling pathways and identify molecules that would be a better therapeutic target.
Methods: We characterize 100 high-grade gliomas by Chromosomal Comparative Genomic Hybridization (cCGH) and determined the incidence of EGFRvIII mutations and amplification, respectively, by RT-PCR and FISH. In 20 tumours, gene expression analysis was evaluated with GeneChip HuGene1.0ST arrays (Affymetrix) and data processed with Partek and Ingenuity Pathway Analysis (IPA) software. Array validation was performed by immunohistochemistry with pY705 STAT3 (Abcam) in 50 of the tumours. **Results:** Concomitant gain of chromosome 7 and loss of 10q, a hallmark of glioblastomas, was found in 83% of the cases. EGFR amplification was observed in 58.3% of the tumours and EGFRvIII mutation detected in 30% of the amplified cases. Gene expression analysis revealed that the most over-expressed genes (FC>10) were genes related with cell cycle control, angiogenesis and cellular invasion and those with the lowest levels (FC<10) were related with neuronal functions (e.g. GABRG2). Functional network analysis of EGFR pathway by IPA defined as hub-molecules: STAT3 and C-MYC. Immunohistochemistry revealed pY705-STAT3-positive tumor cells in all cases.
Conclusion: Our data strongly supports STAT3 as a molecular target in high-grade glioma therapy.

P06.204
Spectrum of STK11 gene mutations in Poland

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Peutz-Jeghersa syndrome (PJS; MIM 175200) an autosomal dominant disorder associated with increased risk of malignances. The occurrence of PJS is estimated from 1/29,000 to 1/120,000 newborn. In most patient the hamartomatous polyps are observed during second or third decade of life. Polyps can be located throughout digestive tract. Risk of colorectal cancer is lower then in FAP or HNPCC, but PJS can be reason of many gastrointestinal discomforts like bowel obstruction, intussusception or bleeding. In Peutz-Jeghers syndrome high risk to development malignancies such as the pancreas, the breast, female and male reproductive organs is observed. Second manifestations are mucocutaneous hypermelanocytic lesions. The pigmentations are usually brown, dark or blue spots developing on lips, hands and feet, in the mucosa of the nose, conjunctiva or rectum appear in about 90% cases. Mutations in STK11 gene are detected in 70 % patients with familial form of PJS and in 30 to 70% sporadic cases of disease. STK11 gene is located on chromosome 19p13.3. Protein coding by STK11 gene is serine/threonine kinase. Loss of STK11 function causes many defects, since STK11 participates in very important cell signaling pathways. Here we present the study considering 30 Polish families with PJS. The STK11 point mutations were detected 9 families and large STK11 gene rearrangements were detected in 6 families.
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P06.205
Levels of acetylated H3 histone in papillary and undifferentiated thyroid carcinoma

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Histone acetylation is a major mechanism to regulate gene transcription. This post-translational modification is modified in cancer cells. In various tumor types levels of acetylation at several histone residues are associated to clinical aggressiveness. By using immunohistochemistry we show that acetylated levels of lysines at

positions 9, 14 and 18 (K9-K14 and K18) of H3 histone are higher in papillary thyroid carcinomas (PTC) than in corresponding normal tissues. Differently, in thyroid undifferentiated carcinomas (UC), only higher levels of K9-K14 acetylation is observed. By using rat thyroid cell lines stably transfected with doxycyclin-inducible oncogenes, we show that the oncoproteins RET-PTC, RAS and BRAF increases levels of acetylated K9-K14 and K18. All these oncogenes activate MAP kinase (MAPK) pathway. The involvement of this signal pathway in the modulation of the H3 acetylation was confirmed by studying the effects of its inhibition. Human thyroid cell line WRO, treated with the MAPK inhibitor PD98059, reduces acetylated levels of these lysine residues. In the non-tumorigenic rat thyroid cell line FRTL-5, TSH increases levels of acetylated K18. However, this hormone decreases levels of acetylated K9-K14. In conclusion, our data indicate that the MAPK signalling pathway and TSH control levels of several acetylated lysines in the context of the H3 histone.

P06.206
Contribution of constitutional *TP53* mutations to Wilms tumour

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Wilms tumour is the commonest renal tumour occurring in childhood and has been reported to occur at increased frequency in Li-Fraumeni syndrome (LFS), a cancer predisposition syndrome predominantly associated with sarcoma, breast, brain and adrenocortical cancers. Constitutional *TP53* mutations are identified in up to three-quarters of families with clinically defined classic LFS. Mutations are typically missense changes at hotspot codons within the DNA-binding domain. We sought to investigate the contribution of constitutional *TP53* mutations to Wilms tumour outside the context of LFS. We undertook mutation analysis of *TP53* in 489 individuals affected with Wilms tumour recruited from Paediatric Oncology centres in the UK. *WT1* and 11p15 defects had been excluded in all cases.

Overall no clearly pathogenic mutations were identified. Two non-synonymous missense variants were identified; V197A and T256A, neither is reported on the IARC *TP53* database and both are predicted to retain at least partial function of transactivation capacity in a yeast assay. Although both are located in the DNA-binding domain they are not at recognised hotspots and we have also observed non-hotspot variants in this domain in a control series (6/1334, p=1.0). Furthermore V197A was present in an unaffected parent and we have observed T256A in a clinical LFS family in which the variant does not segregate with cancer. These data suggest that constitutional *TP53* mutations do not make an appreciable contribution to Wilms tumour susceptibility, outside the context of a family history of LFS.

P06.207
Investigation of expression level and nucleotide variation of the BAX gene in Iranian patients with transitional cell carcinoma of the bladder cancer

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This is a case-control study within investigation of the BAX gene's expression and its nucleotide change in 36 men with transitional cell carcinoma (TCC) and control group (24 healthy men).

Material and method: BAX gene expression in tumoral, adjacent tissues of bladder and peripheral blood lymphocytes of 32 patients and bladder tissue of control group (24 men) is measured by real time PCR. Nucleotide change in promoter region and 6-coding exons is determined by sequencing in 36 patients and control group.

Results: BAX gene expression level in tumoral tissue of 62.5% of patients was higher than adjacent tissue, in 12,5% it was lower and in 25% was equal. In blood cells of 20,83% of patients the level was higher than adjacent tissue's one. SNP polymorphism was observed at 3 locus of this gene.

Conclusion: Bax gene sequence both in peripheral blood lymphocytes and in tumoral cells of patients was homogenous so it is possible to detect nucleotide change only by peripheral blood lymphocytes and to receive information about this gene expression since the significant relation between heterozygosity in promoter region and expression decrease has been clarified in this study.

P06.208
Detection of circulating tumour cells in the peripheral blood lymphocytes of healthy individuals

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INTRODUCTION: The detection of circulating tumour cells(CTC)in the peripheral blood (PB)of cancer patients(pts)has a potential prognostic value.But the early detection of CTC in healthy persons offers more.

AIM OF THE STUDY: Our study analyse the presence and frequency of CTC in the PB of phenotypically healthy individuals(HI).

PATIENTS: During the 2005,450 pts were transferred to Cytogenetic unit for various reasons.Among them there were27pts with breast adenomas,15 with HPV,50with H*p* infection,and 60 with anemia/ leukopenia.

All had a cancerous family history.

With routine blood control,mammography,PAPtest,gastric endoscopy,bone marrow aspiration/biopsy,they had no obvious malignant disease.

METHODS: PB lymphocytes were cultured using standard techniques. ThirtyGTG banded metaphases were analyzed(ISCN2005).Used FISH probes were LSI TOP2A/HER-2/CEP17,EGFR/7,IGH/MALT1, AP12/MALT1,CSF1R,EGR1,D7S486(VYSIS),Hterc/C-MYC/SE 7TC, p53/ATM(KREATECH)

Two hundred interface nuclei were counted for any single probe in all pts once in a year.

RESULTS: From the beginning were CTC ranged 4-10%.Till the end of 2010,we confirmed 5malt gastric lymphomas,4 cervical cancers,9 MDS,and 3 breast cancers.

CONCLUSION: In the absence of manifest clinical disease CTC can persist over a 3-yr period in the PB of HI.

Early detection and quantification may identify people at risk of a malignant disease.It needs careful evaluation and analysis of findings so as to speed up the clinicians on early detection of a forthcoming malignant disease.

P06.209
A multifactorial approach to assess the pathogenicity of unclassified sequence variants in hereditary colorectal cancer susceptibility genes

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Mutations in the mismatch-repair genes MLH1, MSH2, MSH6 and MUTYH are responsible for Lynch syndrome (LS) and MUTYH-associated polyposis (MAP), respectively. About 30% of MisMatch Repair (MMR) and 20% of MUTYH known gene variants are Variants of Uncertain Significance (VUS). Discriminating true pathogenic from benign variants is becoming a major issue in the understanding of the genetic bases of inherited CRC and in the clinical setting.

We undertook a study aimed at defining the significance of 31 VUS (19 missense, 2 silent, 8 intronic, and 2 5'-UTR variants) identified in a series of 188 unrelated probands, referred for either MMR gene sequencing (n = 76) or a suspicion of MAP (n = 112). 6 VUS have been identified in several cases: p.L260R (n = 2) and c.1039-8T>C (n = 2) in MLH1, p.K579K (n = 2), p.L556L (n = 3), c.2005+24T>A (n = 3) and c.1511-9A>T (n = 7) in MSH2.

We evaluated multiple parameters to establish pathogenic significance, including: phenotype of the patients/families in whom the VUS were identified, co-segregation with phenotype, family history, co-occurrence of established pathogenic mutations, evaluation of molecular markers of MMR and MUTYH deficiency in tumour samples, immunohistochemistry analysis for MMR protein expression, frequency in control alleles, in silico prediction of potential effects of the VUS on mRNA and on the encoded protein function. The VUS were classified, when possible obtaining values of posterior probability as recommended by the IARC Working Group on Unclassified Genetic Variants recommendations (Plon et al., Hum Mutat. 2008 29:1282-91).

P06.210
Identification of clonal variations present in tumor through clustering

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Identification of driver mutations in cancer cells is a challenging endeavor. We hypothesize that these driver mutations arise early in the original cancer cells providing it a selective advantage to form distinct clones. Next generation sequencing data provides an opportunity to construct the allele frequency spectrum for all somatic mutations in a particular tumor. Partitioning the allele frequency spectrum in distinct clusters hence can provide an idea of the number of clones present in the tumor cell mass. We here work with the output of tumor sequence data as generated on the 454 platform (Roche Sequencing) and apply clustering algorithm. Initial clusters were obtained using linkage method.To decide upon no. of cluster we compare AIC and BIC and also Gap Statistic.Davis-Bouldin index,Dunn index and some external quality criterion like rand measure, Jaccard matrix were calculated to compare clustering algorithms. Obtaining initial cluster,we propose a methodology to find out the number of clones as well as clonal proportions using EM algorithm. Same algorithm was repeated for normal blood data and tumor data in order to draw something conclusive. Also in each type of data somatic cell vs germline cell new-position mutation vs insertion deletion has been compared.Comparison between number of initial clusters ,clones and their proportions gave rise to some biologically significant inferences.

P06.211***
Exome sequencing of seven melanomas provides new insights in cancer causation and progression, and identifies additional driver gene candidates

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Melanoma is the most dangerous form of skin cancer since it frequently metastasizes. Although a few prevalent mutations have been identified with the sequencing of a single melanoma cell line, the full spectrum of somatic driver mutations remains unknown. We have performed exome sequencing to detect somatic mutations in protein-coding regions of seven melanoma cell lines (five unrelated and two derived from the same patient sampled 12 years apart) and their corresponding germline DNA. The number of coding non-synonymous somatic mutations in the cell lines ranged from 158 to 709. The most abundant class of somatic mutations in all melanomas is C>T, a hallmark of exposure to UV light. In the two melanomas derived from the same patient two thirds of the somatic mutations were shared and showed signature of UV light; remaining mutations might have originated during the transition to the invasive phase. Six melanomas had mutations in the BRAF gene around amino acid 600 and one had a mutation (Q61R) in the NRAS gene. In two melanomas we identified novel mutations in genes that are key regulators of the MAPK pathway. These mutations were also observed in two individuals in a larger sample collection. Remarkably, all cases have germline damaging rare variants in genes involved in the HR and NER pathways. The contribution of these variants to predisposition to melanoma is under investigation. Such results will shed light on the natural history of tumour development.

J06.01
Role of metabolic pathways Genes in tumorigenesis and tumor progression

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Maintaining normal body status is controlled by at least three important metabolic cycles that closely regulate synthesis of amino acids and nucleotides. Normal cells use different sources of energy including glycogen, fatty acids and amino acids, but the key energy source is glucose for cell growth. Instead, tumour cells show unusual metabolic pathways with significant re-programming. Cancer cells need a lot of metabolic energy for their rapid proliferation, so a non-oxidative energy source is used such as glucose. This re-programmed activity underlies events in the physiology of tumour cells and sometimes generates harmful products such as reactive oxygen species or oxygen free radicals, and sometimes results in unequal storage of nucleotides for DNA synthesis that increases the mutation rate. Cancer cells have high rates of glycolysis and NADH-oxidation that generate a lot of ROS. Re-programming of genes involved in metabolic pathways is a sign of physiological changes in cancer cells. Expression of some genes is altered dramatically in different stages of tumour growth that directly control metabolic pathways. These changes are characteristic of tumour cells. They help tumour cells to become more aggressive. The genes involved in oncogenesis include *GLUT1*, *G6PD*, *TKTL1* and *PGI*/*AMF* in the glycolytic pathway, *ACLY*, *FAS* and *ACC1* in lipogenesis and *RRM1*, *RRM2*, and *TYMS* for synthesizing nucleotides. Over expression of these genes in many cancer cells was observed. Taken together, down-regulation of these genes can inhibit tumorigenesis and tumour progression.

J06.02
Genetic basis of Rhabdomyosarcoma

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Rhabdomyosarcoma is a cancer of cells which normally develop into skeletal muscles, most common in children under the age of 10. A specific chromosomal abnormality, t(2;13)(q35;q14), was reported in many cases of advanced rhabdomyosarcoma patients by RT-PCR, FISH and spectral karyotyping. The other frequent abnormalities observed were rearrangements of chromosome 1p and trisomy of chromosome 8 using conventional cytogenetic techniques such as G banding. The translocation between chromosome 2 and chromosome 13 causes the movements of the gene called PAX3 (or PAX7 if it's chromosome 1) right next to a gene called FKHR. Paired box (Pax) genes are a family of tissue specific transcription factors containing a paired domain and usually a partial or complete homeodomain. The PAX genes play an important role in causing cells to grow while an embryo's muscle tissue is being formed, but these genes usually shut down once they're no longer needed. The normal function of the FKHR gene is to activate other genes. Moving them together likely activates the PAX genes, which may lead to the formation of tumor. Complete trisomy 8 is a leading cause of miscarriage in women while infants with trisomy 8 mosaicism are likely to survive but with retarded psychomotor development and moderate to severe mental retardation. About 3% of all tumors are found to be rhabdomyosarcomas, with them being rare in teenagers and adults but constituting more than 50% of embryonic tumors making them detectable by chromosomal analysis during pre-natal diagnosis.

J06.03
Breast cancer is the most common cancer in young Iranian women

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Breast cancer is one of the multifactorial diseases in which both genetic and environmental factors are involved in the aetiology. Various factors have been identified as playing an important role in stimulating this cancer. During the last decades the increasing incidence of breast cancer has made it the most prevalent cancer among young Iranian women. The mean age of onset of breast cancer in Iranian women is about 10 years lower than that of their counterparts in developed countries.
A new study of the prevalence of breast cancer in immigrants from Asian countries to Sweden suggested that there are some biological factors that caused early onset of breast cancer in immigrants, including Iranian women, compared to native women of Sweden. Epidemiological aspects of Iranian breast cancer are uncertain and unfortunately no study has specifically addressed breast cancer risk factors in young Iranian women up to now. The purpose of this study was to investigate the influence of polymorphic variants defined by single nucleotide polymorphisms (SNPs) in the displacement loop (D-loop) region of

mitochondrial DNA (mtDNA) on the risk of developing breast cancer in young Iranian women. We used PCR and RFLP analysis in this study. Based on our results we detected a significantly greater incidence of the mtDNA polymorphisms T239C and A263G in early onset of breast cancer patients and we think this may be associated with an increased risk of breast cancer in young Iranian women.

J06.04
Gene mutations (K-ras) in pancreatic cancer and chronic pancreatitis patients in north indian population

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Background/Aim: To understand the genetic alterations of K-ras gene in North Indian patients with pancreatic diseases, and the possible correlation between the presence of mutation, histopathological findings and other risk factors are assessed by standard questionnaire. **Methods:** A total of 65 pancreatic cancer patients, 50 chronic pancreatitis and 50 controls were inducted in the study. K-ras mutations were examined using the PCR RFLP, followed single-strand confirmation polymorphism (SSCP) and automated DNA sequencing. **Results:** K-ras mutational analysis in surgically resected tissue samples of the pancreatic carcinoma was 72.30 %(47/65) which was significantly higher than the chronic pancreatitis 6% (3/50) (P<0.01). It was also found that K-ras mutation rate was progressively increased from normal duct at the tumor free resection margin to pancreatic carcinoma. The mutation pattern of Kras codon 12 observed in pancreatic carcinoma was GGT_GGA and this was identical in chronic pancreatitis also. In this study male showed more prevalence of K-ras mutations than female. The age > 40 yrs showed statistically higher incidence of pancreatic cancer than chronic pancreatitis (P<0.05*). Smoking was found to be one of the risk factor for pancreatic cancer compared to chronic pancreatitis (p<0.05*). Other possible associated risk factors like alcohol consumption, diabetes, tea, coffee, Milk products and dietary habits were found to be statistically non-significant. **Conclusion:** This study shows evidence of K-ras gene mutation to be statistically higher in pancreatic cancer compared to chronic pancreatitis and this suggests that K-ras gene represent a critical early step in the genesis of pancreatic cancer.

J06.05
Similarity of DNA methylation profile in tissues with benign breast disorders and lymph nodes metastasis in patients with breast cancer

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Despite many works examining the individual gene methylation in various malignancies, the question about the specificity of the methylation profiles of the large groups of genes in the carcinogenesis dynamics remains open. This study was aimed to assess the DNA methylation profile in tissues with benign breast disorders (BBD), breast cancer (BC) and lymph nodes metastasis (LNM). We analyzed 6 samples with BBD, 19 samples with BC, 5 samples with LNM, and 6 control histologically normal epithelium samples from women with BC using Illumina GoldenGate Cancer Panel I (Illumina, USA). Cluster analysis identified a group of samples with a high similarity of DNA methylation profile. This group included all tissues with BBD and LNM (except one) and one sample from each of the BC and control groups (p<0.001). The t-test revealed 261 CpG sites responsible for the combined clustering of these samples (FDR<0.01). All other samples with BC and morphologically normal epithelium were not clustered and showed a high heterogeneity of the DNA methylation profile. The relatively common DNA methylation profile in the samples with BBD and LNM may indicate a significant impact of epigenetic modifications into early origin of cell clones with BBD and LNM phenotypes in breast cancer. This study was performed using biological samples from the Integration Project of the Siberian Branch of Russian Academy of Medical Sciences for the breast cancer research.

J06.06
Chromosomal abnormalities in follicular thyroid carcinoma

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Follicular carcinomas are the second most common thyroid cancers; the mortality being decided by the degree of invasion. A left hemithyroidectomy specimen was observed and it was reported that the section showed a nodule composed of cells arranged in diffuse microfollicular pattern and macrofollicles. The cells were cuboidal with moderate cytoplasm and oval uniform vesicular nuclei. Nuclear crowding was observed and the neoplasm showed molecular focal capsular invasion. Deletion of a part of chromosome 22q was reported. Recent studies have mapped tumor suppressor loci associated with meningioma and neurofibromatosis type 2 (NF2) to 22q. The deleted region reported has been expected to consist of the region corresponding to that of the tumor suppressor genes associated with NF2 and meningiomas. In this case, t(2;3)(q13;p25) translocation and the PAX8-PPAR gamma1 fusion was also expected to be present. Rap1, a member of Ras family, has been implicated in the regulation of oncogenic pathways in thyroid and it has been found to regulate the ERK cascade and it is specifically required for the RET/PTC-induced activation of BRAF-MEK-ERK. Rap1 functions as a molecular switch which cycles between inactive GDP-bound and active GTP-bound forms. Rap1GAP, a GTPase activating protein, functions as negative regulator of Rap1 activity. It was also reported that loss of Rap1GAP function may be implicated in promotion of invasion in follicular thyroid carcinoma as the tumor was found to be minimally invasive. The study of extent of loss of Rap1GAP activity can be used to determine the degree of invasiveness of the tumor.

P07 Cancer cytogenetics

P07.01
Rabl's Orientation Today: Implications for Diagnostic Genetics, Epigenetics and Aetiology of Chromosome Aberrations

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Rabl's orientation of chromosomes - all centromeres polarized to the centrosome, while the telomeres flutter at the opposite pole - is a time-tested phenomenon. Moreover a wealth of observations in plants and animals indicate that the chromosomes are probably distributed in two groups, each representing one of the two parental genomes (Kikuchi S et al., Genes Genet Syst 82(5): 369, 2007; Chaudhuri et al., Anticancer Res. 28:3573, 2008).

Here we present our observations predominantly made on blood and bone marrow cells which match to the scheme of maternal and paternal chromosomes forming two different groups, both in interphase and metaphase. Cytologic findings about the centrioles in relation to their ploidy status and this order of chromosomes in two groups suggest a more detailed appearance of Rabl´s orientation.

The polar distribution of the centromeres of all chromosomes towards the centrsome results obviously from each chromosome of one of the two sets being tethered to one of the two centrioles in the centrosome of a diploid cell. On the basis of this phenomenon genomic karyotyping may be feasible which determines the parental origin of acquired chromosome abnormalities, contributes to understanding the origin of the aberration itself by tracing the right ontogenetic development and genealogic lineage and may facilitate studies on epigenetics (Chaudhuri et al., Cellular Oncology 27:327, 2005).

P07.02
HR-CGH and array CGH analysis of acute myeloid leukemia patients with normal or complex aberrant karyotype by G-banding.

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Acute myeloid leukemia (AML) is the most common type of leukemia among the elderly. Lymphocytes of approximately 55% of AML patients show various chromosomal aberrations, including structural and numerical alterations. Such changes may not only correlate with morphological and clinical data, but also serve as prognostic factors.
In our study we analyzed bone marrow samples of 40 AML patients with normal (10 patients) or complex (30 patients) karyotype by conventional cytogenetics. We then assessed the genomic changes by the means of high resolution comparative genomic hybridization (HR-CGH) and microarray CGH method. Genomic losses were found more frequently than gains. The most frequent losses affected 7q (30%), 3p (20%) and 5q (18%), and the most frequent genomic gains included 11q (30%) and 21q (20%).
In patients with initially normal karyotypes it was possible to determine single or multiple aberrations with aCGH. In this group losses were also more frequent than gains. Changes detected using array CGH were verified by the analysis performed with FISH technique in all cases.
The number of aberrations found by means of aCGH was significantly higher as compared with GTG results, which indicates that aCGH enables more sensitive karyotype examination and may be used in the search for new prognostic and predictive factors in AML.

P07.03
Balanced translocations in myelodysplastic syndromes.

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INTRODUCTION: Myelodysplastic syndromes (MDS) are a group of clonal haematopoietic stem cell diseases characterized by cytopenia(s), dysplasia in one or more of the major myeloid cell lines, ineffective haematopoiesis, and an increased risk of developing acute myeloid leukaemia (AML). Chromosomal abnormalities are detected in half of patients with the novo MDS, with losses and gains of genetic material being the most frequent cytogenetic findings. Balanced translocations are not frequently associated with MDS. Recurrent translocations associated with MDS are the t(3;21)(q26.2;q22.1),t(5;12)(q33;p13), t(3;12)(q26;p13),t(11;16) (q23;p13.3),t(6;9)(p23; q34).
OBJECTIVE: To identify the frequency of balanced translocations in patients diagnosed with MDS in our centre.
PATIENTS AND METHODS: Four hundred and twenty cytogenetic studies were performed on bone marrow samples from patients diagnosed with MDS.
RESULTS: Twenty seven balanced translocations were diagnosed in 23 (5,4%) patients, who were classified according to the WHO (2008) criteria: refractory anaemia (AR) (n=1), refractory cytopenia with multilineage dysplasia (n=7), AR with excess of blasts-1 (n=8), AR with excess of blasts-2 (n=5), and chronic myelomonocytic leukaemia (n=1). Seventeen of the 27 (63%) translocations were found as a part of a complex karyotype (>=3 chromosomal abnormalities). Two new translocations (2;3)(p21;q26.2) and t(13;17)(q21;p13), were found in two patients. All chromosomes were involved in the translocations, with the exception of chromosomes 8, 18, 21 and Y.
REMARKS: The infrequency of balanced translocations in MDS makes their identification and reporting imperative for the recognition of the recurrent ones. This increases the likelihood of identifying which genes are possibly involved in the pathogenesis of the neoplasia.

P07.04
BACs On Beads™ technology, an alternative to CGH array for tumor analysis

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Objective : BACs On Beads™ (BoBs) (Perkin Elmer, Turku, Finland) has been recently validated by our team showing its robustness and versatility for evaluating chromosome abnormalities in pre-natal diagnostic. In BoBs technology the Bacterial Artificial Chromosomes are immobilized onto encoded beads that are used to assay gains and losses. We reported here our preliminary study on 12 breast tumor DNA from frozen tissue. Methods : We evaluate here the Karyolite, a new kit which could screen all abnormalities with 3 to 4 beads per chromosome. 3 BACs are encoded per beads to increase the specificity. 12 breast tumors DNA were extracted with phenol chloroform from frozen tissue. Karyolite BoBs was performed at Poissy hospital using a L200 Luminex and 44k Agilent's CGH array at Institut Curie-René Huguenin. Results: Out of those 12 tumors DNA, no false positive result in BoB was found according to CGH array analysis. At the opposite, and as suppose by the beads design, small chromosome abnormalities as segmental duplications or deletions were not retrieved. All large chromosome abnormalities (one to half of a chromosome arm) were identified without any ambiguity. Conclusions : For tumor analysis, BoBs technology could open very interesting outlets as it is cheaper, less time consuming (100ng of DNA), low material consumption, and rapid throughput. Position in diagnosis is still under discussion as only low number of locus are explored. However, this could be proposed as an alternative to CGH array with dedicated specific beads design to breast cancer chromosome abnormality.

P07.05
Investigation of Human Papillomavirus and Biomarkers of Disease Progression and Therapy in Cervical neoplasia

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Cervical cancer represents the fifth highest female cancer mortality rate and second most common occouring malignancy worldwide. While curable in early stages, the prognosis for advanced stage disease is poor and mortality rates remain high. We aim to evaluate and compare the expression of various proteins/genes as markers of progression, from early stage neoplasia right through to invasive carcinoma. Primary investigations will involve the use of a wide range of biomarkers including diagnostic, prognostic and therapeutic markers, as well as identifying human papillomavirus status/type in correlation with abnormal expression patterns. Samples will include biopsy material from normal, low grade, high grade and invasive neoplasia.
In particular we will be investigating the epithelial growth factor receptor (EGFR), as its over expression/activation has been identified as a common feature of multiple cancer types and contributes to epithelial tumour growth. This will be evaluated through the use of specific antibodies directed against various domains of the receptor, as well as family dimerization partner human epidermal growth factor receptor 2 (HER2) and downstream effector proteins (AKT, p-AKT and mTOR); involved in up regulation of mRNA translation, cellular growth, proliferation and survival (anti-apoptosis). In addition to IHC, fluorescent insitu probes directed against the EGFR gene will allow for identification of increased gene copy number which contributes to accelerated tumour growth. Analysis of these biomarkers may yield information of use in predicting whether specific tyrosine kinase inhibitor (TKI) chemotherapies directed against EGFR, could prove to be an effective future therapy for invasive cervical cancer patients.

P07.06
Indication for CDKN2A mutation analysis in familial pancreatic cancer-families without melanomas

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Background: Carriers of a CDKN2A-gene mutation run a high risk of developing melanomas. In addition, they have an additional increased risk of developing pancreatic cancer (PC). Familial pancreatic cancer (FPC) patients with a personal or family history of melanomas are therefore offered CDKN2A-mutation analysis. On the other hand, CDKN2A testing is not generally recommended in the literature in FPC families without a history of melanomas.

Methods: FPC families were defined as families with clustering of PC and not meeting diagnostic criteria of specific other familial cancer syndromes. Medical details of cancer diagnoses were retrieved and reviewed. Blood samples were obtained for DNA isolation from PC-patients or first degree relatives (FDR) and analyzed for mutations in CDKN2A.

Results: Among 40 FPC families, DNA analyses were carried out in 28 families (70%), leading to identification of CDKN2A-mutations in six families (21.4%) None of the CDKN2A families fulfilled the diagnostic criteria of familial atypical multiple mole melanoma (FAMMM) syndrome and in three CDKN2A families no melanomas and/or dysplastic nevi were observed. In these latter three families two different CDKN2A-mutations were found; in two families the Dutch founder mutation p16-Leiden (c.225_243del, p.Ala76fs) and in the third family the c.19_23dup, p.Ser8fs-mutation. After disclosure of the CDKN2A-mutation in one of the families, a curable melanoma was diagnosed at subsequent dermatological surveillance in a 17-year old female family member.

Conclusion: CDKN2A-mutation analysis should be included in genetic testing in FPC families, even in the absence of reported melanomas. This strategy will enhance the recognition of individuals at risk for PC and facilitate the early detection of melanomas.

P07.07

The initial experiences with detection of hTERC and MYCC genes in HPV positive cells in cervical carcinoma and cervical intraepithelial dysplasia using Vysis Cervical FISH Probe Kit

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It is known that this cervical cancer develops from precancerous intraepithelial neoplasia (CIN) which are characterized by series of genetic abnormalities. The progression of cervical intraepithelial dysplasia to cervical carcinoma has been associated especially with the genomic integration of oncogenic human papilloma virus (HPV) and gain of the human telomerase genes hTERC (3q26) and c-MYC (8q24).

Recently, Vysis Cervical FISH Probe Kit was designed to identified HPV infected cells and determine copy number of the hTERC a c-MYC genes via fluorescence *in situ hybridization* in cervical cytology specimens.

In our work, we have studied genetic aberrations on a series of cytological specimens obtained from patients of Department Gynaecological Oncology of Masaryk Memorial Cancer Institute Brno with cervical carcinoma or proven precarcinoma (CIN2/CIN3). The patiens were divided into two groups.

Using classical I-FISH gain of hTERC gene was found in 8 (42 %) of 19 patiens with cervical carcinoma, all woman with hTERC gain were HPV positive and 63 % of them showed positive lymphovascular invasion.

In 15 patiens we tested gain of hTERC and MYCC genes in HPV positive cells using new Vysis Cervical FISH Probe Kit and relationships between HPV infection, chromosomal alterations and diagnosis were analysed.

Our work confirmed that gain of hTERC is significantly associated with cervical carcinoma and worse prognosis. Furthermore we conclude that Vysis Cervical FISH Probe Kit can be used as effective diagnostic procedure to identity the patients carrying highly risking HPV infection and chromosomal aberrations associated with this malignancy.

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P07.08

Oligonucleotide microarrays uncover new aberrations in Chronic Lymphocytic Leukemia not previously identified by standard cytogenetic methods

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Chronic lymphocytic leukemia (CLL) is the most commonly diagnosed leukemia in the western world and is highly variable, with life expectancies ranging from months to decades. Traditional cytogenetic studies have provided genetic insight into the disease, leading to prognostic significance and treatment for individual patients. Microarray technology has made significant impact in the assessment of constitutional genetic disorders, and this same application has the capacity to enrich the understanding of hematologic malignancies. In this study we show the utility of an oligonucleotide array designed for the detection of copy gains and losses found in leukemia and lymphoma. We performed microarray assessment of 35 samples with a working diagnosis of CLL. Ten had previous chromosome analyses. The average number of abnormalities revealed by banding was 4, whereas the average number of abnormalities revealed by microarray analysis was 6.5. Additional information not seen by karyotype and fluorescence *in situ* hybridization (FISH) was uncovered in 31% of cases. Novel findings were identified, including losses and gains of susceptibility loci on 11q24, 8p21, 4p16p15 and 5q35. Our results support microarray analysis as a diagnostic tool for identifying cytogenetic abnormalities that are associated with CLL. Further use of microarrays in CLL will likely identify recurrent novel aberrations, which may prove to be new treatment targets.

P07.09

Molecular cytogenetic study of derivative chromosome 9 deletion in 336 Tunisian chronic myeloid leukemia patients

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The Philadelphia chromosome (Ph), produced by the t(9;22)(q34;q11.2) is the cytogenetic hallmark of chronic myeloid leukemia (CML). Deletions on derivative chromosome 9 (der (9)) may occur with t(9; 22) and could influence the disease progression.

The aims of this study are to investigate the frequency of derivative der (9) deletion in Tunisian patients with CML, and to assess the correlation between this deletion and the cytogenetic response for patients treated with hydroxyurea (HU) or imatinib (IM). Karyotype analysis of 336 patients with CML was performed with R-banding. Fluorescence in situ hybridization was carried using home brew probes 17L7 and 248J22 for detecting respectively adjacent 5'ABL and 3'BCR deletions on der(9).

Cytogenetic study demonstrated typical t(9;22)(q34;q11) translocation in 89,6% and variant translocation in 10,4% of patients. Interphase-FISH studies showed deletion of der(9) in 59 (17,6%) of the 336 patients, 23 (39%) of them had variant rearrangements. There are 19 patients with solely 5'ABL deletion and 40 with concomitant 5'ABL and 3'BCR deletions. Cytogenetic response was evaluated during 18 months with HU or IM therapy.

Our results demonstrate that: (a) all patients with 3′BCR deletion have 5′ABL deletion, (b) 5′ABL and 3′BCR deletions arise simultaneously with Philadelphia chromosome formation, (c) deletions on der(9) chromosome were frequently encountered in older patients and in patients presenting variant rearrangements, (d) both 5'ABL and 3'BCR

deletions were associated with cytogenetic response failure in patients treated with HU , although patients treated with IM and carrying deletions on der(9) presented better cytogenetic response.

P07.10
Clonal evolution in Chronic Lymphocytic Leukaemia

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INTRODUCTION Chronic lymphocytic leukaemia is characterised by variable clinical course. The most frequent chromosomal abnormalities are trisomy 12, deletion of 13q14.3, 11q22.3 and 17p13; each of these is a relevant prognostic factor. These abnormalities may appear at diagnosis or during follow-up.
The goal of this study is to determine the frequency of clonal evolution in CLL patients. Clonal evolution (CE) is defined as newly acquired aberration during the disease course.
MATERIAL AND METHODS One hundred and twenty-five patients with CLL were analysed by conventional and/or molecular cytogenetics (FISH) at diagnosis and during follow-up. Two samples from the same patient were studied after a minimum follow-up time of 6 months after the first genetic study. The median time between diagnosis and follow-up was 38 months (range 6-116).
RESULTS 30% of the patients (21/71) showed CE using conventional cytogenetics. 57% of the patients with normal karyotype at diagnosis showed chromosomal abnormalities during follow-up. Twenty-three of 53 patients (43%) had CE by FISH; in 17 of them, FISH abnormalities were observed at diagnosis and new chromosomal aberrations were acquired during disease course.
CONCLUSION Clonal evolution is a significant event in CLL patients. The most frequent abnormality which appeared during disease course was del(13q). The median time to follow-up for CE was 10 months. Conventional and molecular cytogenetics were necessary to detect clonal evolution in CLL patients. We believe that CE could be a new prognostic factor and an indicator of change in clinical course of the disease.

P07.11
Emergence of karyotypically unrelated clone during complete remission of acute monocytic leukemia

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AML is a heterogeneous group of disease. Besides morphological and cytogenetic heterogeneity, of interest are different types of remissions. One form of remission is characterized by repopulation of the marrow by normal cells, and the other by clonal cell proliferation. The data on clonal remission are limited, and some 40 cases have been reported so far. The frequency of clonal remission is variable and range from 1% to 12% of acute leukemia. Nature, mechanisms of origin and clinical significance of clonal cell proliferation at remission are not elucidated yet. We present the results of clinical and cytogenetic investigation in a girl with AML and unrelated clonal chromosome aberration during remission and literature review of published data on clonal remission in patient with acute leukemia. Our patient is 3½-old-girl referred to the hospital with hepatospenomegaly, hematomas and polymicroadenia. Cytomorphologic analysis revealed AML-M5A. Chromosome study at diagnosis showed abnormal clone with 48,XX,+21,+mar and at remission following induction therapy a normal karyotype was observed. Eleven months after diagnosis, while the patient was in first complete remission an unrelated abnormal clone with t(1;18) was observed. This report highlights the importance of cytogenetic study in patients follow up and detection of unrelated clonal rearrangement during remission of acute leukemia.

P07.12
Fluorescence In Situ Hybridization on peripheral blood for BCR/ABL fusion gene detection is a robust alternative method for evaluation of minimal residual disease in CML.

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CML is the first malignancy for which a specific chromosomal abnormality was described - Philadelphia chromosome. Monitoring of minimal residual disease in CML by detection of Philadelphia chromosome is an important part of response evaluation of patients in treatment with tirozinkinase inhibitors, because it allows early detection of suboptimal response or resistance to treatment.
Materials and Methods: We analyzed 25 bone marrow specimens using interphase FISH and compared the results with those of conventional cytogenetics and with interphase FISH on peripheral-blood specimens.
Results: in comparison to conventional cytogenetics - interphasic FISH on peripheral blood did not generated any discrepancies in percent of BCR/ABL positive nuclei.
Conclusion: Fluorenscent in situ hybridisation is a molecular cytogenetics technique which allows minimal residual disease monitoring on interphase nuclei. MRD monitoring on peripheral blood has the advantage of generating results in short time (even in 24h) and is less invasive than bone marrow biopsy. In comparison with bone marrow culture it has the advantage of being a direct method which abolish artifacts introduced by the culture. On the over side - it has disadvantage of being able to identify only specific chromosomal malformations - Philadelphia chromosome in this case, all other important aberrations in disease progression being omitted. For the use of this method in MRD there is a need for large standardization studies, present clinical standard being conventional cytogenetics. In this study we analyzed FISH techniques as a rapid and uninvasive alternative method for MRD monitoring in CML patents undergoing tirozine-kinase inhibitor treatment.

P07.13
Genome-wide analysis of recurrent copy number abnormalities and copy neutral-loss of heterozygosity in colorectal cancer

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Two forms of genetic instability have been observed in colorectal cancer (CRC): chromosomal instability, that is present in 85% cases and is characterized by structural and numerical chromosomal abnormalities (aneuploidy); and microsatellite instability (MSI), characterized by a deficiency of the mismatch repair system and associated to a normal or quasi-normal karyotype. Recent advances in molecular cytogenetics techniques are likely to introduce a revolution in the field. In the present study we analysed a series of 57 CRC samples and their related normal mucosae by high resolution genomic arrays (Affymetrix SNP 6.0 arrays) and set up a series of bioinformatics tools that allow the generation of a rapid report on broad (>25% of a chromosomal arm) and small somatic copy number abnormalities (CNAs), somatic homozygous deletions, focal high level amplifications, and regions of loss of heterozygosity (LOH). MSI tumors represented 14% of our CRC series and showed a median values of somatic broad CNAs of 3 (range 1-6), while microsatellite stable (MSS) tumors showed a median value of 12 (range 0-24). Therefore all MSI tumors were below a threshold of 6 CNAs, while 18% of MSS were below this threshold, representing a group of microsatellite and chromosomal stable tumors. No correlation was observed between the number of tumor associated CNAs and the number of somatic copy neutral-LOH regions, suggesting that different mechanisms underlie such chromosomal abnormalities. Analysis of recurrent CNAs identified somatic homozygous deletions that cannot be ascribed to regions of inherent fragility.

P07.14
Genomic imbalances identified by array-CGH in endometrial cancers

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Background: Endometrial cancer is the third most common female malignancy in Europe. While most patients with early stage disease have a good prognosis, there are women who will recur as endometrial carcinoma is the third cause of gynecologic cancer death. But it is sometimes difficult to determine the clinical prognosis with classical histo-pathological factors. Previous studies on endometrioid adenocarcinomas using Comparative Genomic Hybridization (CGH) showed large chromosomal alterations with gain in 1q, 19p, 19q, 8q, 10q and 10p; and losses in 4q, 16q and 18q.

Material and Method: The aim of this study was to investigate chromosomal alterations by array-CGH to identify discrepancies in two different groups of tumors and hyperplasia, in relation to grade, stage and clinical data. Tissue specimens were obtained from 37 patients (31 neoplasms and 6 hyperplasia) and array-CGH analysis was performed using genomic DNAs extracted from frozen specimens.

Results: Copy number variations (CNV) were detected in 24 cases of neoplasm (77 %) but not in hyperplasia. On average, four CNV by case were observed with a 1:1 ratio between amplification and deletion. The most frequent alterations were trisomy for chromosomes 7 and 8, gains for segment 1q and losses in regions 9p, 11p,16q and 17p. We found two amplifications and two deletions for 10q and 11q regions.

Conclusion: These preliminary data need to be confirmed by further investigations in order to establish new significant prognostic factors and the specific chromosomal regions affected by copy number changes will be interesting to investigate in further genetic studies.

P07.15

Genomic instability at common fragile site *FRA14B* in cancer

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Common fragile sites (cFS) are chromosomal regions prone to breakage under conditions partially inhibiting DNA synthesis. Approximately 90 cFSs have been cytogenetically identified, but few of these have been completely characterized. Most characterized cFSs coincide with extremely large genes and hotspots of chromosomal rearrangements in cancer. We localized an aphidicolin-inducible cFS, *FRA14B*, to an 800kb region of the 14q23 chromosome band using six-color FISH mapping with BAC probes on metaphase chromosomes of aphidicolin-treated lymphocytes. *FRA14B* overlaps the large gene encoding gephyrin (*GPHN*, 670kb), a protein involved in synapse development and molybdenum cofactor biosynthesis. To assess the possible role of *FRA14B* in generating cancer-associated rearrangements, we performed oligonucleotide array CGH on 150 cell lines and primary tumors. We detected multiple breakpoints within this cFS in 13 cancer genomes of melanoma and glioma cell lines and neuroblastoma, colon cancer and breast cancer cell lines and primary tumors. The breakpoints clustered at the first intron of *GPHN* and extra-genic regions, but not within exons. Subsequent validation of CGH results using FISH revealed at least two apparently normal copies of *GPHN* per genome in the majority of tumors and cell lines bearing rearrangements. All deletions were limited to introns or located within additional ectopic copies of *GPHN*. *GPHN* transcripts were detectable by RT-PCR in all the cell lines. In summary, activation of newly-mapped cFS, *FRA14B*, can cause the instability of *GPHN*, the role of which in cancer development remains to be investigated.

P07.16

Investigation of chromosomal aberrations in hepatocellular carcinoma patients as detected by fluorescence *in situ* hybridization

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Hepatocellular carcinoma (HCC) is a very common and highly malignant tumor, associated mainly with chronic viral hepatitis, cirrhosis of any cause, aflatoxin exposure and ethanol consumption. Cytogenetic analysis on HCC has been limited because of poor hepatocyte growth in vitro. Conventional cytogenetic studies have demonstrated frequent abnormalities of specific chromosomes in HCC. Molecular cytogenetic approaches have been applied only rarely in the characterization of HCC. The main aim of this study was to evaluate genetic aberrations of different chromosomes in HCC. The study included 45 patients with HCC, who have been diagnosed and treated at National Cancer Institute, Cairo University, Egypt.

Interphase cytogenetics by fluorescence in situ hybridization with the use of a panel of centromere-associated DNA probes for chromosomes 1, 4, 8, 9, 13, 17, 20 and Y were performed on paraffin-embedded HCC specimens.

The most common chromosomal aberrations detected were gain of chromosomes 8 in 12 cases (34.28%), 17 in 6 cases (17.14%). Loss of chromosome Y was detected in 6 of male cases (30%). Monosomy 4 was also detected in 5 cases (14.28%). Negative correlation could be detected only between chromosome 4 and 8. ($r = -0.381$, $P < 0.05$). Correlations between gain or loss of chromosomes and the different clinicopathologic parameters in the patients investigated, indicated negative correlation between: chromosome Y and age and chromosome 1 and cirrhosis.

Gains and losses of DNA found in this study probably involve oncogenes and tumor suppressor genes that play a role in the puzzle of hepatocarcinogenesis.

P07.17

ERBB2 (Her-2/neu) Amplification Status Determination by Copy Number Variation Analysis

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Gene copy number variations by DNA amplification are a genetic alteration that can lead to deregulation of gene expression, and eventually contribute to neoplastic transformation. Amplification of the *ERBB2* gene abnormality is known to influence breast cancer (BC) prognosis and response to therapies. Currently, Her-2/Neu protein product expression and/or gene amplification are usually detected by Immunostaining or by molecular techniques such as fluorescence in situ hybridization (FISH). Here, we determined Her-2/Neu amplification status on 45 clinical research BC samples using TaqMan® Copy Number Assays, and previously characterized with immunostaining and FISH methods. A side-by-side testing on the Applied Biosystems® 7900HT Fast Real-Time PCR System and the Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument was performed to evaluate data reproducibility between the two systems. Our results demonstrate that the results obtained with the TaqMan® Copy Number Assays were highly concordant with those previously reported by FISH, on both the Applied Biosystems® 7900HT Fast Real-Time PCR System and the Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument. Therefore, we demonstrate that the Copy Number Real Time method can be an easy, robust, and economically advantageous method for Her-2/Neu amplification status evaluation. We conclude that the Applied Biosystems® 7500 Fast Real-Time Dx Instrument is an ideal instrument for clinical laboratories that require not only technically reliable and economically feasible methods, but also routinely controlled instruments. TaqMan® Copy Number Assays and 7900HT Fast Real-Time PCR System are for research use only, and not intended for any animal or human therapeutic or diagnostic use

P07.18

The assessment of ErbB2 gene over expression in respectable gastric Cancer and its Relationship with histopathology subtype, grading and stage of disease

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Background: ErbB2 gene, also called Her2, is located in chromosome 17 (17q12-q21) and encodes a 185-kd transmembrane tyrosine kinase receptor (p185), which is a member of the epidermal growth factor receptor family. Amplification of the ErbB2 gene and overexpression of the ErbB2 protein have been observed in various solid tumors, including breast and gastric carcinomas. We aimed to evaluate the ErbB2 content in respectable gastric cancer in this country and its relationship with clinicopathologic parameters of tumors.

Methods: This study was a prospective analysis of 100 specimens

from 100 patients with respectable gastric carcinoma which were admitted in three hospitals in this country. Indirect immunostaining was used to evaluate the expression of this receptor in formalin-fixed paraffin-embedded tissue sample.
Result: ErbB2 amplification was present in 26(26%) of 100 gastric carcinoma. ErbB2 was more common in the intestinal type of gastric cancer (33%) than in the diffuse (5%) or the mixed type (0%) (p=0.001), also ErbB2 was more common in well-differentiated gastric cancer than other grade (p=0.001), but it was not associated with gender, age at diagnosis or clinical stage.
Conclusion: ErbB2 amplification is common in intestinal type and well-differentiated gastric carcinoma but there was no correlation between ErbB2 expression and tumor stage. The relatively high percentage of ErbB2 expression positive tumors will provide a useful target for immunotherapy of these cancers.

P07.19
Cytogenetic characterization of pediatric mixed phenotype acute leukemia with complex karyotype

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Mixed phenotype acute leukemias (MPALs), defined by the 2008 WHO classification, are rare leukemias in which blasts express antigens of both lymphoid and myeloid lineages. Furthermore MPALs are partitioned according to the two cytogenetic abnormalities reported quite frequently in the affected patients, namely rearrangements leading to *BCR-ABL1* fusion and translocations involving 11q23 and *MLL* gene. Karyotype abnormalities have been documented in about 80-90% of MPAL pediatric patients, with no uniquely associated specific chromosomal aberration. Here we report on a case of childhood MPAL showing a complex karyotype, characterized by M-FISH and array-CGH. The patient was a 5-year-old girl diagnosed with MPAL (T/myeloid) based on the expression of T-lymphoid cyCD3 and myeloid MPO antigens. Cytogenetic analysis by Q-banding technique on peripheral blood and bone marrow blasts revealed a hypodiploid karyotype with structural changes involving chromosomes 1, 9, 12, 13, 16. To characterize the rearranged chromosomes, M-FISH analysis was performed. Further array-CGH using 5500 BAC clones (565 Kb median resolution) showed partial losses of 1p, 9, 13q, 16q. The gain of chromosome 12p was also evident. Overall, based on the combined results of Q-banded mitoses, M-FISH and array-CGH, it was possible to define the karyotype as: 45,XX,der(1)t(1;12)(p36.12;p13.2) t(1;12)(q12;p13.2),-9,der(12)t(1;12)(q12;p13.2)t(12;13)(q24;q14.3), der(13)t(12;13)(q24;q14.2), der(16)t(9;16)(q31.1;q21). The combination of conventional cytogenetic techniques with M-FISH and array-CGH has allowed a careful analysis of complex chromosome alterations. The analysis of a larger series of MPAL patients could help to identify diagnostic and/or prognostic markers of this rare type of leukemia.

P07.20
Genomic screening of hyperdiploid subgroup of multiple myeloma patients using oligo-based arrayCGH technique

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Multiple myeloma (MM) is the second most common hematological malignancy. It is
characterized by malignant transformation of clonal proliferation of B-lymphocytes and their
accumulation in bone marrow There are two major genetic subclasses of MM: a hypediploid variant associated with the incidence of chromosome number changes, mainly trisomies of chromosomes 3,5,7,9,11,15,19 and 21, and a non-hyperdiploid variant associated with high incidence IgH locus translocations. and thus with poor disease prognosis.
Main aim of this study was to perform genomic screening of hyperdiploid subgroup of MM patients using oligo-arrayCGH technique focusing on finding new prognostic markers for MM diagnosis.
We analyzed 67 bone marrow samples. DNA was obtained from CD138+ cells separated by MACS or FACS techniques.
Taking together, hyperdiploidy (defined in MM as extra copy of chromosomes 5, 9, or 15 together in karyotype) was found in 48% (31/65 patients). Most common chromosomal changes involved trisomies of chromosomes 3,5,7,9,11,15,19,21 (incidence >50%). Gain(1q) was observed in 40% of H-MM cases (12/31). Most common loss was 13,6 MBp minimal deleted region (MDR) in 8pter-8p22 (25%, 8/31), -13 (23%, 7/31) and del(16q) (20%, 6/30). In one case, we observed biallelic deletion of *TRAF3* involved in MM pathology through deregulation of NF-kappaB pathway and amplification of *SALL1*, transcriptional repressor supposed to be a part HDAC complex.
Array-CGH provide us powerful tool for characterization of another genomic changes important for diagnosis and classification of the development of this hematological malignancy.
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P07.21
Case report of a rare karyotype in a patient with multiple myeloma

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Multiple myeloma (MM) is a malignancy disorder characterized by the proliferation of a single clone of plasma cells (PC) derived from B cells in the bone marrow. In this tumors there is a high genetic instability resulting in a wide variety of genetic and chromosomal abnormalities. Cytogenetics abnormalities are considered an important prognostic indicator and are observed in 30-50% of the cases. The most frequent are monosomy or deletions of chromosome 13, translocations involving the immunoglobulin heavy chain locus (IGH) and ploidy status.
The authors present the results of a cytogenetic study in a seventy three years old male patient with MM.
Bone marrow cell culture and GTL banding was done according to protocols in the laboratory. Cytogenetic analysis was performed following the standard cytogenetic guidelines. FISH painel (13q-, 17p-, t(4;14), t(11;14)) was applied.
The karyotype was: 47, XY, +8, t(6;19)(p21.3:p13.3), t(11;14)(q13;q32)[16] / 46, XY [3]. FISH was positive to t(11;14) (88%).
In this patient we found a characteristic anomaly in MM, confirmed by FISH, a trisomy 8 that is a rare event in lymphoid neoplasia and a translocation not described in the literature. FISH is a sensitive method to investigate cytogenetics of MM but it should be performed always with conventional cytogenetics analysis. They are important to predict treatment responses and prognosis.rte superior do formulário
The authors compare the cytogenetic findings and the patient's clinical status with those described in the literature.

P07.22
Translocation (x;20)(q13;q13.3) in a patient with myeloproliferative disorder: Case report and review of the literature

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Rearrangements involving the X chromosome are rare in hematological malignancies. Only a few recurrent translocations involving the X and different autosomes have been associated with hematological malignancies.
In contrast, structural alterations of the long arm of chromosome 20 are non-random recurring abnormalities in patients with myelodysplastic syndromes (MDS), myeloproliferative disorder (MPD) and acute myeloid leukemia (AML). This suggests the loss of function of tumor suppressor genes at this location.
In this study, we describe an additional case of t(X;20)(q13;q13) in a myeloproliferative disorder (MPD). To the our knowledge, this is the fith case of MPD associated with this rearrangement.
The patient was an 75-year-old female presenting with a thrombocytosis at 1.600.000/mm³. The bone marrow evaluation suspected a MPD of type essential thrombocytemia. Cytogenetic studies performed on bone marrows cells demonstrated a homogeneous karyotype with a

t(X; 20) (q13.q13.3) as the sole abnormality.
In total, the translocation t(X;20) has been reported in nine previous cases : 4 MPD, 3 MDS and 2 AML patients. It is invariably found in old women (mean age of 69 years), which suggests a probable influence of epigenetic factors. We will discuss the possible implication of the inactive X in the translocation, which may exercise a position effect on 20q. The silencing of 20 q genes may be involved in the clinical presentation of myeloid disorders especially as deletions 20q represent one of the most frequent chromosomal abnormalities seen in myeloid disorders. Our case further confirms the recurrent character of the t(X;20)(q13;q13) in myeloid malignancies.

P07.23
Monitoring DNA damage in Tunisian hospital Staff using cytokinesis block micronucleus method and chromosomal aberration test

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Introduction: Hospital staff is exposed to many compounds which are suspected to be genotoxic and carcinogenic. Micronucleus test (MNs) and chromosomal aberrations test (CAs) are an excellent means to evaluate the genotoxicity and the risk associated with theses releases. Our study reports the finding of these genotoxicity tests for hospital staff exposed to ionizing radiation (RI), anti-cancer drugs and formaldehyde (FA) from Farhat Hached Hospital of Sousse (Tunisia).
Patients and Methods: Assessment of chromosomal damage was carried out in peripheral lymphocytes of 20 nurses handling anti-cancer drugs, in 31 exposed to FA, and in 45 exposed (RI). We used controls matched for age, gender and life style.
MNs frequency was evaluated after cytokinesis block. A total of 1000 binucleated cells were scored for each subject. Mitomycin C was used for detection of CAs.
Results: The frequency of binucleated micronucleated cells was significantly higher in all exposed groups when compared with controls ($p < 0.05$). The frequency of CAs was also significantly higher in exposed than in controls. For example, in nurses handling anti-cancer drugs, the frequency of MNs was 9.4‰± 2.88 versus 4.36‰± 1.32 in controls. In CA analysis, we detected in exposed group a significant increase (about 5.7 fold) of CAs.
Conclusion: Based in the fact that exposure to MNs and CAs are an excepted markers of exposure to genotoxic substances, our finding of higher frequency of CAs and MNs in peripheral lymphocytes of exposed workers indicates that FA, anti-cancer drugs and (RI) are a mutagenic agents in humans.
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P07.24
Usefulness of complex genomic profiles for the differential diagnosis of osteosarcomas mimicking aneurysmal bone cyst.

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Aneurysmal bone cyst (ABC) is a rare primitive lytic bone lesion. Translocation of the *USP6* locus occurs in over 60% cases leading to its overexpression. Although classical forms are characteristics on radiological & histological levels, some cases remain difficult to distinguish from a telangectatic osteosarcoma (OS).
We report 2 cases of a lytic lesion of the proximal humeral epiphyso-metaphysis in a 16 yr-old boy (pat1) & in a 13 yr-old girl after a traumatism (pat2). On both, a first biopsy showed an aspect of ABC. The X-Ray confirmed the evolution of the lesion. A second surgical procedure consisted of curettage, phenolisation, allograft and plating. Additional curettages were performed in patient 2. However, 4 months & 5 years after the ABC discovery, respectively for pat1 and pat2, the histology was in favour of malignancy.
Complex clonal chromosomal alterations were detected from the first specimens of both cases in 4-27% cells. Clonal evolution was further observed: duplication of the initial clone in both cases associated with the acquisition of *CDKN2A*/9p21 & *TP53*/17p13 deletions (pat1) & of *CDKN2A*/9p21 deletion and increased amplifications of *MDM2*/12q15 locus on a giant chromosome (pat2). No *USP6* rearrangement was detected in both cases.
The detection of complex genomic alterations, specially tumor suppressor loci deletions or *MDM2* amplification gave strong arguments in favor of a malignancy, confirmed on the surgical specimen. Genomic data, even in a very few proportion of cells, were useful to reach finally the diagnosis of OS on limited specimens where no clear criteria were present.

P07.26
A case of Chronic Myelogenous Leukaemia Ph+ and Splenic Marginal Zone-B Cell Lymphoma with t(6;14)(p21;q32)

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Chromosomal translocation t(6;14)(p21.1;q32.3) appears mainly in Multiple Myeloma and Plasma Cell Leukaemia, but it has also been reported in Diffuse Large B-Cell Non-Hodgkin Lymphoma and Marginal Zone B-Cell Lymphoma.
We report a case of a 65-year-old patient who was diagnosed with possible Chronic Myelogenous Leukaemia, which was confirmed by cytogenetic and molecular studies: 46,XY,t(9;22)(q34;q11)[7], ratio bcr-abl/abl p210 (84%). After being treated with Gleevec®, the study of his bone marrow showed a 55.7% of mature lymphoid cells, (54.1% B-lymphocytes). Cytogenetic studies then showed two cellular lines: 46,XY,t(9;22)(q34;q11)[14]/46,XY[2], and a decrease in the ratio bcr-abl/abl p210 (5.3%). Eleven months after the first analysis, the t(9;22) could only be detected by molecular studies; however, an additional cellular line was present when lymphocytic line was stimulated: 45,X,-Y,t(6;14)(p21;q32)[13]/46,XY[21]. Fluorescence In Situ Hybridization (FISH) for RB1, TP53, ATM and centromere of chromosome 12 were normal. The patient was then diagnosed with a Chronic Myelogenous Leukaemia + Splenic Marginal Zone B-Cell Lymphoma. Cytogenetic follow-up analysis demonstrated an increase in the cellular line with t(6;14). FISH studies using a LSI IgH dual color, break apart rearrangement probe, showed that 52% of the cells had the IgH translocation with loss of the telomeric part of the probe. A decrease in the t(6;14) line was observed after treatment with splenectomy and additional bcr-abl p210 studies indicated that the patient was in major molecular response.
Our report contributes to the clinical and cytogenetic description of the rare association between Splenic Marginal Zone B-Cell Lymphoma with t(6;14) and Chronic Myelogenous Leukaemia.

P07.27
Derivative (6)t(1;6)(q21;p21): a recurrent cytogenetic abnormality in two cases of pediatric therapy-related myelodysplastic syndrome

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Childhood myelodysplastic syndromes (MDSs) are a rare group of clonal hematopoietic stem cell disorders occurring de novo or secondary to cytotoxic chemotherapy and/or radiotherapy for a previous malignancy. Cytogenetic aberrations have been detected in about 50% of de novo MDS and in almost all therapy-related MDS (t-MDS) patients. The most common abnormalities are complete or partial loss of chromosome 7 and trisomy 8. Unbalanced rearrangements involving the long arm of chromosome 1 and leading to its trisomy, usually as t(1;7), are also recurrent. We report two cases of pediatric t-MDS with der(6)t(1;6)(q21;p21). This unbalanced translocation has been reported in the literature associated with adult chronic myeloproliferative disorders. The first patient developed refractory cytopenia 9 years after suspension of chemo- and radiotherapy for a medulloblastoma diagnosed at the age of 7 months. The second patient, a 6-year-old boy, is slowly developing MDS features after a diagnosis of neuroblastoma 4S at age 1 month and of anaplastic lymphoma 10 months later. Cytogenetic analysis of peripheral blood and bone marrow blasts of both patients revealed der(6)t(1;6)(q21;p21). FISH for the painting of chromosomes 1 and 6 confirmed the rearrangement. Further array-CGH analysis performed on the bone marrow of the second patient, using 5500 BAC clones, showed

gain of material of chromosome 1 (q21.1->q44) and chromosome 6 (p22.1->p12.1) and loss of material of chromosome 6 (p25.3->p22.1). Array-CGH analysis is being carried out in the other patient to verify whether the imbalance is identical.

P07.28
Characterization of new rearrangements of cell line HT1376. FISH contribution.

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Tumor cell lines are valuable preclinical models in oncological studies concerning human cancer, potential treatment modalities and pharmacologically studies.

Human cancer urothelial cell lines represent important tools in the study of bladder cancer, particularly in evaluating the chemoprevention of different compounds and the therapeutic effect of anticancer drugs on apoptosis and cell proliferation.

Although the karyotypic aspects of some cell lines have sometimes been taken into account, the cytogenetic studies are usually limited to classical methods. Documenting their chromosome composition adds valuable genetic data and offers a reference to evaluate genomic changes that may occur *in vitro*.

HT1376 is an invasive transitional carcinoma cell line used frequently in bladder cancer research. Established in 1977 and diagnosed as stage 2, grade 3 this cell line has a karyotype of high complexity and is not yet characterized cytogenetically.

In this work, we have attempted to cytogenetic characterize the HT1736 bladder carcinoma cell line by a combination of classical cytogenetics analysis (GTL, NOR and CBG banding) and fluorescence *in situ* hybridization (subtelomeric, painting, alpha satellite and unique sequence probes). We also evaluate the degree of DNA fragmentation of the cell line and in normal urothelial cells as a control.

Using this approach, we have identified several novel rearrangements that could reflect useful molecular markers involved with the development of bladder cancer. They can be important in improving prognostic on tumor diagnosis, and to develop new therapeutic approaches.

J07.01
Investigation of *FANCA* mutations and their effects on the expression of *FANCA* in Iranian patients with Fanconi anemia

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Fanconi Anemia (FA) is an autosomal instability syndrome characterized by progressive bone marrow failure (anemia),developmental anomalies (various congenital malformations), high cancer susceptibility (acute nonlympocytic leukaemia) and cellular hypersensitivity to cross linking agents such as diepoxybutane (DEB) and mitomycin C (MMC). The patients display lots of phenotypic variations. At least 13 genes and corresponding complementation groups are underlying the disease. Eight of the FA proteins (FANCA, -B, -C, -E, -F, -G, -L and -M) and other components assemble in a nuclear complex, the FA “core complex”.In this study we aimed to screen mutations in FANCA gene in Iranian patients diagnosed with fanconi anemia. Chromosomal breakage analysis with MMC was performed to establish diagnosis of FA in 40 Iranian patients. RNA was extracted from the blood and consequently cDNA was synthesized. The cDNA of each patient amplified with 22 set PCR reaction. The size of the amplicons were between 200 and 250 bp. Each PCR product screened with SSCP (Single Strand Conformational Polymorphism) and HRM (High Resolution Temperature Melting Curve) for mutation detection. The aberrant SSCP band or HRM was sent for direct sequencing. In this presentation we will demonstrate our data regarding FANCA mutation frequency among Iranian FA patients. Furthermore, we will present some of the new mutations found in this group of FA patients

J07.02
The radiation-induced aberrations of chromosomes in lymphocytes of breast cancer patients

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Aim. To investigate cytogenetic effects in peripheral blood lymphocytes (PBL) of breast cancer patients exposed in vitro to γ-irradiation in the low dose range with regards to individual radiation sensitivity.

Material and Methods. The irradiation of lymphocytes isolated from healthy donors was carried out on therapeutic apparatus “Rockus” (with 60Co source) in range of 0,1-1,0 Gy. The metaphase analysis of chromosome aberrations had been carried out with group kariotyping including G2-radiation sensitivity assay.

Results. Background frequency of chromosome aberrations in PBL of breast cancer patients was 7.2±0.95/100 cells and was 6-fold higher than in the cjntrol group of healthy donors. The peculiarity of spontaneous aberrations spectrum consist in the emergence of dicentric chromosomes (0.16/100 cells) in 55% of examined patients. When the PBL culture was irradiated in the low dose range (0.1-0.5 Gy), the dose curve had an abnormal shape with the formation of the plateau (dose-independent part). The boundaries of its location depends on the individual radiation sensitivity of patients, the second plateau could be formed with increase of individual radiation sensitivity.

Conclusions. For the first time, it was shown that individual radiation sensitivity significantly affects the yield of radiation-induced chromosome aberrations in PBL of breast cancer patients and this effect was dependent on radiation dose. This dates are advisable by radiation therapy of early forms of breast cancer.

J07.03
Examination of Numerical Chromosome Aberration in ALL by FISH Technique

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Leukemia is a malignant disease characterized with uncontrolled proliferation by loosing the ability to differentiate of hemotopoietik cells. Acute lymphoblastic leukemia (ALL) occurs with the abnormal uncontrolled and excessive proliferation of lymphoblast. ALL is responsible for approximately 80% of childhood leukemia and approximately 20% of adult leukemia. Many numerical and structural abnormalities play an important role in the risk assessment and the diagnosis of ALL. Numerical anomalies are in the form of chromosomal losses and gains. Losses and gains of some chromosomes are more common than the other chromosomes. In current study, numerical changes in chromosomes 8, 10, 11, 17, 18, X and Y were determined in 30 ALL patients by using FISH technique. Chromosomal changes were observed in all patients as monosomy X in 12 patients (40%), disomy X in all 18 male patients (100%), trisomy X in 16 patients (53%), tetrasomy X in 4 patients (13%), loss of Y in 2 patients (11%), disomy Y in 14 patients (78%), monosomy 8 in 29 patients (97%), trisomy 8 in 26 patients (87%), tetrasomy 8 in 3 patients (10%), monosomy 10 in 22 patients (73%), trisomy 10 in 9 patients (30%), monosomy 11 in 27 patients (90%), trisomy 11 in 8 patients (27%), tetrasomy 11 in 1 patient (3%), monosomy 17 in all patients (100%), trisomy 17 in 9 patients (30%) and monosomy 18 in 19 patients (63%). These analyzes of numerical abnormalities in patients with ALL provide useful data for diagnosis and prognosis.

P08 Statistical genetics, includes Mapping, linkage and association methods

P08.01

MMP-13 (-77A>G) gene polymorphism and susceptibility to abdominal aortic aneurysm or aortoiliac occlusive disease

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Objective: Abdominal aortic aneurysm (AAA) and aortoiliac occlusive disease (AIOD) are common vascular disorders considered to be complex diseases with both genetic and environmental risk factors involved. Involvement of various matrix metalloproteinases is very important in the pathogenesis of these diseases. Matrix metalloproteinase-13 (MMP-13) was shown to be up-regulated in AAAs and atherosclerotic lesions of aorta. A functional MMP-13 promoter polymorphism (-77A>G) was found to be associated with functional status in rheumatoid arthritis. The aim of the present study was to examine if MMP-13 (-77A>G) gene polymorphism is a susceptibility factor of AAA or AIOD in Polish patients. Results: Based on a PCR-RFLP analysis the MMP-13 genotypes (A/A; A/G/; G/G) were determined in three selected groups: 300 patients with AAA and 312 patients with AIOD who underwent surgery; 313 individuals from control group. Genotypes were compared with demographic and clinical data of subjects and analyzed in relation to risk factors. No significant differences in genotype distribution and allele frequencies of MMP-13(-77A>G)gene polymorphism in the study groups were found. Conclusion: This study found no evidence of association of MMP13 (-77A>G)gene polymorphism with AAA or AIOD in Polish patients.

P08.02

A study role of the Alu-insertion of the gene ATP-binding cassette transporter ABCA6 in lipid metabolism

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INTRODUCTION: We studies gene, encoding ATP-binding cassette transporter ABCA6 is located on the chromosome 17 (17q24.2-3) consist of 38 exons and 39 introns. Alu-element Ya5_435 localized in the 25 intron of the gene ABCA6. ABCA6 involved in ATP-dependent transport of various molecules through biological membranes, and supports the lipid homeostasis of the body, localized on the surface of macrophages.

MATERIALS AND METHODS: We studied samples DNA of 318 individuals in the age of 18-65 years living in Republic Bashkortostan and them parameters of lipid profile. The analysis genetic polymorphism is realized by polymerase chain reaction (PCR). Assessing the impact of Alu-insertion in a gene ABCA6 on lipid metabolism was performed by ANOVA.

RESULTS: We found that the genotype ABCA6 *I /*I leads to a decrease in the level of high density lipoproteins (HDL), and allele ABCA6*D - an increase (F = 5.196; ρ = 0.023). Genotype ABCA6*D/*D leads to an increase in triglyceride (TG) levels, and allele ABCA6*I - to decrease (F = 7.242; ρ = 0.008). Thus, the Alu-insertion into ABCA6 is associated with a low level HDL and TG of the person.

P08.03

APEX microarray method in personalized medicine of chemotherapeutic treatment

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Drug response in patients who underwent chemotherapy is accompanied by many adverse effects. The prediction of therapeutic effect may vary depending on individual gene variability. Many studies focus on the association between phenotype and DNA variations in genes of interest. Comprehensive approach in genotyping of numerous variants is needed to achieve improvements in personalized medicine such as selection of appropriate drug for treatment.

This work focuses on the design of a pharmacogenetically relevant panel which could be applied for the rapid genotyping of patients treated with methotrexate, thiopurines, 5-FU, irinotecan or taxanes prior to treatment as well as in studies searching for new genotype-phenotype associations. We selected 97 variable positions in 36 genes which were previously associated or may be potentially involved with altered drug response. Of these, 94 SNPs were genotyped by APEX microarray method, which is a valuable tool for fast, reliable and cost-effective typing. Variations of tandem repeats or gene deletions were genotyped by capillary electrophoresis and PCR detection, respectively. Direct sequencing served as reference method for validation of APEX chip and control of observed results. 300 DNA samples from unrelated healthy volunteers were tested to estimate genotype frequencies for a Slovak population, which may be helpful in estimation of central European frequencies. All data were checked for H-W equilibrium and genetic linkage between loci and haplotype analysis were also performed.

P08.04

Autosomal recessive intellectual disability: homozygosity mapping identifies 27 single linkage intervals, at least 14 novel loci and several mutation hotspots

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With a prevalence of around 2% intellectual disability (ID) is a frequent cause of severe disability. Significant excess of ID in the progeny of consanguineous matings and functional considerations suggest that autosomal recessive forms of ID (ARID) must be relatively common. To shed more light on the causes of ARID, we have set out in 2003 to perform systematic clinical studies and autozygosity mapping in large consanguineous Iranian families with non-syndromic ARID (NS-ARID). Previously we have reported 12 novel ARID loci as well as 2 causative gene defects in this cohort. By tripling the size of our cohort we were able to identify 27 additional unrelated families with NS-ARID and single-linkage intervals; 14 of these define novel loci for NS-ARID. Altogether, 13 out of 39 single linkage intervals observed in our cohort were found to cluster at 6 different loci on chromosomes 1p34, 4q27, 5p15, 9q34, 11p11-q13 and 19q13, respectively. Five of these clusters consist of two significantly overlapping linkage intervals, and on chr 1p34, three single linkage intervals coincide. We showed by Monte-Carlo simulation that the probability for this distribution to be due to chance is only 1.14*10(-5). Thus, in contrast to our previous conclusions, here we indicate that common molecular causes of NS-ARID do exist, and in the Iranian population, the most frequent ones may well account for several percent of the patients. Mutation screening by next generation sequencing in order to find the underlying gene defects at these loci is ongoing.

P08.05

Linkage analysis in 147 Iranian families with Autosomal recessive non-syndromic hearing loss

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Hearing impairment is the most common sensory disorder and affects about 1 of every 1000 newborns. In developed countries at least 50% of the cases are due to genetic defects most often resulting in non-syndromic deafness (70%), of which autosomal recessive inheritance predominates (80%). Although hearing loss is very heterogenous, mutations in GJB2 gene (at DFNB1 locus) are the major cause of autosomal recessive non-syndromic hearing loss (ARNSHL) in many populations. Our previous study showed that mutations of GJB2 gene do not contribute to the major genetic load of deafness in the Iranian population.

To determine the other important loci in ARNSHL in our population, 147 deaf Iranian families with 2 or more affected members, who were negative for GJB2 gene mutations, were subjected to linkage analysis using flanking or intragenic STR markers for 34 loci.

Until now, 59 families have been linked to 18 loci: 9 families to DFNB4, 8 to DFNB3, 4 to DFNB24, 4 to DFNB21, 4 to DFNB7/11, 4 to DFNB2, 3 to DFNB28, 3 to DFNB29, 3 to DFNB37, 3 to DFNB42, 2 to DFNB16, 2 to DFNB30, 2 toDFNB31, 2 to DFNB49, 2 to DFNB59 and one family for each of 4 loci including: DFNB6, DFNB9, DFNB18, DFNB53. Mutation analysis of the corresponding genes revealed several novel mutations. In conclusion, DFNB1, DFNB4, DFNB3 and DFNB2 are the most prevalent loci in ARNSHL in Iranian population. Linkage study for remaining families and mutation screening for the rest of linked families, are underway.

P08.06

DFNB93, a novel locus for autosomal recessive moderate-to-severe hearing impairment

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Introduction: Hearing impairment (HI) is the most common sensory defect in humans. Autosomal recessive non-syndromic HI (ARNSHI) is linked to over 70 chromosomal loci, with about half of the corresponding genes identified. Novel loci and their genes can increase our insight into the mechanisms of HI. While the phenotype of the majority of families is severe to profound, DFNB21 *(TECTA)* has been described to show a recognizable milder audiometric profile. Presented here is a novel locus in an Iranian family with moderate to severe ARNSHI, localizing to a new locus on chromosome 11q12.3-13.2. **Methods:** Linkage to DFNB21 (*TECTA*) as well as 14 other known loci was ruled out. Illumina 6K SNP chips (Linkage IVb panel) were used for a genome scan, followed by fine mapping by microsatellite markers. **Results:** Genome wide linkage revealed a multipoint LOD score of 3.51 on 11q12.3-13.2. The homozygous linkage interval is flanked by the markers D11S1765 (60.68 Mb) and D11S1975 (70.30 Mb) spanning a 9.62 Mb interval. The name DFNB93 was assigned to this locus by the Human Genome Organization (HUGO) Nomenclature Committee. **Conclusion:** The new DFNB93 locus partially overlaps with the DFNB63 locus. However, it does not include the responsible *LRTOMT* gene. Furthermore, DFNB63, unlike DFNB93, accounts for severe to profound HI. DFNB93 is the second ARNSHI locus with a distinctive audiometric profile. The sequencing of coding regions and exon-intron boundaries of several candidate genes including *LRTOMT*, *CFL1*, *KCNK4* and *RELA* revealed no disease causing variants.

P08.07

Analysis of MDR1 (ABCB1) gene polymorphisms in Behcet's Disease

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Behcet's disease (BD) is a chronic multisystem disorder. The cause of BD remains a mystery; infectious agents, immune mechanism, and genetic factors are implicated in the etiopathogenesis of the disease, which remains to be explained.

The human MDR1 gene encodes a P-gp (P-glycoprotein) plays a key role in determining drug bioavailability, and drug response exist amongst different populations.

In this study, we were investigated genotype and haplotype distribution of MDR1 gene polymorphisms in BD patients, and genetic differences from the control group. Three MDR-1 genetic markers (C1236T, G2677T/A and C3435T) were analyzed in 97 BD patients and 107 unrelated Turkish subjects. The genotyping analysis was performed by PCR-restriction fragment length polymorphism (RFLP) methods. Statistically analysis of data was performed by using Arlequin 3.1.1 and SPSS 16.0 Softwares.

Our result showed that, genotypic and allelic frequencies each three separated SNP in MDR1 gene is not associated with BD (p>0.05). In the haplotype study, we have found two haplotypes may have be protective to BD. T-G-T haplotype were found 4.7 % in control but were not found in BD patients (p=0.002). The other haplotype C-T-T was detected 7.0 % in control and 2.2 % in BD patients (p =0.019; odds ratio, [OR], 0.28; 95% confidence interval [95% CI], 0.09-0.86). Our study was showed that MDR1 gene and their polymorphisms may be associated with BD in Turkish patients.

P08.08

Next generation sequencing from raw data to mutation report

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Next Generation Sequencing is knocking on the door of diagnostics laboratories which are still reluctant to handle such a large amount of data. Although various software packages have been developed, both open and closed source, for the research and bio-informatics community, none of them really addresses all the specific needs of the diagnostics community, namely:

User-friendliness: use-ability by non bioinformaticians

Patient centric database system

Variant consequence prediction support

Flexibility to analyze data from various equipments

Connectivity to public and private databases

We present here the first version of Gensearch®NGS, a framework to analyze, visualize, interpret and manage NGS data in a patient centric database. The framework integrates all steps from quality control to alignment, variant detection, visualization and interpretation support (UMD Predictor, Human Splice Finder, dbSNP,□) up to reporting, both internally and publicly to the Gen2Phen Café Rouge. Gensearch®NGS is built around the concept of plug-ins, allowing use of either proprietary tools for alignment and variant calling or any major public tool (e.g. BWA, Stampy, Bowtie, VarScan). The framework hides the complexity of those tools. Also integrated is a tool to visualize the alignments in a fast, efficient and user friendly way, it integrates data from external databases, like Ensembl, as well as variants found on any other patient from the user database. The framework also integrates the possibility to use Cloud Computing through POP-C++ and POP-Java programming tools to distribute alignment tasks and, therefore to improve performances. Gensearch®NGS has been developed and validated within the FP7 NMDchip project.

P08.09

Association study between Coronary Artery Disease and rs1333049 and rs10757274 polymorphisms in 9p21 locus in Khuzestan province

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Introduction: Coronary Artery Disease (CAD) is a Multifactorial and Heterogenic disease. In CAD atherosclerosis plaques have been formed in internal wall of coronary artery. That reducing coronary blood flow may lead to Myocardial Infarction. Many genes and loci, such as the 9p21 locus are believed to be involved in the etiology of CAD. Two polymorphisms of 9p21 locus, rs1333049 and rs10757274 have shown remarkable association with CAD in several studies.

Methods: We collected blood samples from 170 CAD patients and 100 healthy persons as control from Hospitals in Ahvaz**.** Association of rs13333049(C/G) and rs10757274 (A/G) polymorphisms with CAD was evaluated using Tetra ARMS PCR technique.

Results: In rs1333049 polymorphism frequencies of CC, CG and GG genotypes in patients were respectively 18.2%, 65.3% and 16.5% and in control cases were 25%, 67% and 8% respectively. GG Genotype in rs1333049 polymorphism was found in CAD patients more than control cases (OR: 0.354, 95%CI= 0.138-0.912, P=0.032).

In rs10757274 polymorphism frequencies of AA, AG and GG genotypes in patients were respectively 8.2%, 58.3% and 33.5% and in control cases were 35%, 63% and 2% respectively. GG Genotype in rs10757274 polymorphism was found in CAD patients more than control cases (OR: 0.014, 95% CI: 0.003 -0.065, P value: 0.0001).

Conclusion: The rs1333049 polymorphism shows a weak association with CAD but rs10757274 Polymorphism has significantly associated with CAD. These variants may help in identification of patients with increased risk for coronary artery disease.

P08.10

CFHR5 mutation screening in sporadic patients with macroscopic hematuria or unknown etiology nephropathy

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Our team contributed to the molecular investigation of a new autosomal dominant renal disease seeming to be endemic in Cyprus, which is characterized by microscopic and synpharyngitic macroscopic hematuria, renal failure (especially in males) and C3 glomerulonephritis. Affected individuals were found to have an internal duplication of exons 2-3, of the complement factor H-related protein 5 (*CFHR5*) gene. Results from 105 Cypriot patients carrying this mutation show that 41% of male patients over 50-yo develop end stage of renal disease.

No other mutations have been reported in *CFHR5* gene in relation to C3 glomerulonephritis or other glomerulonephritis cases. We screened 20 sporadic cases with macroscopic hematuria or unknown cause nephropathy, for mutations in the CFHR5 gene. We used SURVEYOR endonuclease digestion, which identifies and cleaves at mismatched base-pairs in heteroduplexes. The exons and flanking intronic regions of *CFHR5* were amplified with appropriate primers. If cleavage was evident at the digested PCR product (agarose electrophoresis), DNA re-sequencing was performed.

Four variants were identified (5UTR+249TC, P46S, IVS1+17TA, IVS5+33TG), but their frequencies found through 200 chromosomes of the general population, did not differ from the patients cohort. P46S and IVS5+33T>G (novel SNP) were also genotyped in additional 38 unknown glomerulonephritis and 39 unknown nephropathy patients but again the SNPs frequencies were comparable with the ones of the general population, therefore they cannot be associated with a pathogenic effect. It is probable that some patients carry large rearrangements or gross indels that cannot be easily identified with the method we used.

P08.11**

Human *cis*-regulatory SNPs (*cis*-SNPs) altering transcription factors binding and gene expression

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Up to 30% of RefSeq transcripts are regulated by *cis*-SNPs leading to phenotypic differences. These variants can be identified by mapping differences in allelic expression (AE) on Illumina1M/2.5M BeadChips. Mapped *cis*-rSNPs explain >50% of population variance in AE.

Our systematic approach to isolate causal *cis*-rSNPs includes integrative analysis of AE-mapping data in CEU and YRI LCLs with the intersection of common SNPs (1000 Genomes) and functional non-coding elements (wgENCODE) catalogs, along with ChIP-seq assays and assessing promoters and enhancers at high coverage.

Fine-mapped *cis*-rSNPs are enriched in regulatory elements and show predominant localization to 5' transcription start site. Reporter gene assays validated 62% of promoter *cis*-rSNPs. Integrative genomic approaches can successfully isolate causal *cis*-rSNPs beyond promoters: *rs909685* is strongly associated with differential AE of *SYNGR1*, shows association by RNA-seq. in LCLs, is located in active chromatin (DNaseI HS), and shows an allelic H3K4me1 signal in our ChIP-seq. analysis. The allelic enhancer activity at this site was verified by reporter gene assays, underscoring the capacity of population genomic approaches in revealing function and variation of non-coding sequence elements. Multiple other sites show converging functional data suggesting specific mechanisms for common *cis*-rSNP action. However, despite numerous functional genomic datasets, only a subset of mapped *cis*-rSNPs yield straightforward hypotheses, suggesting the need for further tools for assessing non-coding DNA. Additional information can be derived from our comparisons of *cis*-rSNPs observed in LCLs with those from other cell lineages and will guide how to more comprehensively enumerate causal *cis*-rSNPs in the human genome.

P08.12

Genome Wide Association Study of Left Ventricular Phenotypes - ASCOT-HACVD genetic study

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Background: Alterations in left ventricular (LV) structure and function provide independent prediction of myocardial infarction and stroke. These echocardiographic traits are moderately heritable (~20-40%). The aim of this study was to identify genetic variants associated with these LV prognostic markers across the whole human genome.

Methods: 885 hypertensive participants in the Hypertension Associated CardioVascular Disease (HACVD) sub-study of the Anglo-Scandinavian Cardiac Outcomes Trial underwent echocardiography 1.5 and 3.5 years post randomization to BP and lipid lowering therapies. The average value of four measures of LV structure and function were studied; LV hypertrophy (LVM/height$^{2.7}$, LVMi), diastolic dysfunction (e'), systolic function (ejection fraction) and LV filling pressure (E/e'). 318,000 Single Nucleotide Polymorphisms (SNPs) were directly genotyped (Illumina HumanCNV 370 Duo BeadChip), and approximately 3.5 million loci were imputed using IMPUTE from Hapmap rel#24 reference. Poorly imputed SNPs were excluded from further analysis. GWAS was performed using linear regression analysis under an additive model, adjusted for cardiovascular risk factors, and for principal components representing major genetic variation within the study population.

Results and Discussion: None of the directly genotyped SNPs showed significant associations with any of the LV phenotypes. By contrast a number of regions containing imputed SNPs with low minor allele frequencies (<2%) were significantly associated with LVMi. Permutation testing indicated that these SNPs were significant after correction for multiple testing of both genotypes and phenotypes. It is difficult to distinguish whether these associations reflect unmeasured genetic stratification in the population or direct causation by chromosomal regions. Hence replication studies are underway.

P08.13

Multivariate association analysis of arterial calcification in the Nelson Study.

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Atherosclerosis is a major cause of death in the industrialized world. Although the relation between calcium deposits and atherosclerotic plaques is well known, little is known about the genetic basis of calcification. We studied common genetic variation in the NELSON study for a role in aorta and coronary artery calcification.
The Nelson study is a prospective cohort for early detection screening of lung cancer. We analyzed genome-wide SNP genotype data collected in 3082 participants with multiple chest CT scans. We developed a novel image-processing algorithm to quantify levels of calcification in the aorta and the coronary arteries.
We compared three methods in order to find genetic loci associated with coronary artery calcification and aorta calcification (R^2=0.13): a polygenic model, cross-trait meta-analysis, and multivariate regression. Although no single SNPs reach genome-wide significance (p<5E-08), polygenic analysis shows that coronary artery and aorta calcification share a substantial genetic background. The explained variance increases when more SNPs are included, reaching approximately 13% for both traits when SNPs with P-values of up to 0.05 are included in the model.
These results demonstrate that large-scale genome-wide meta-analyses of calcification may reveal important genes involved in forming calcium deposits.

P08.14
Genetic polymorphisms in CYP17 and AR genes and the risk of premature coronary artery disease

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Background: Gender differences and sex hormones are known to influence the risk of developing Coronary Artery Disease (CAD). Several genes are involved in the synthesis or function of sex hormones. Two of them which play a crusial role during the human sex steroidogenesis or function are the cytochrome P450c17α gene (CYP17) and the androgen receptor gene (AR). The aim of this study was to investigate the effect of two single nucleotide polymorphisms (SNPs) of these genes in developing premature CAD.
Methods: A total of 370 Greek cohorts were investigated for the genetic variations T/C of the promoter of the CYP17 and G/A of the AR. The patient group consisted of 230 CAD patients as documented by coronary angiography, aged less than 58 years, while 150 healthy individuals served as controls. All cohorts were genotyped by the PCR-RFLP method.
Results: Statistical analysis indicated no significant differences in genotype or in allele frequencies between the patient and the control group, for both the studied polymorphisms.
Conclusion: The genetic variations T/C CYP17 and G/A AR, could not be a useful genetic markers for premature CAD in our Caucasian population.

P08.15
A Genome wide linkage analysis on large Coronary Artery Disease family suggests linkage to chromosome 19

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Coronary artery disease (CAD) is the most common type of heart disease and the leading cause of death worldwide in both men and women. A positive family history is a recognized cardiovascular risk factor. We identified a family with multiple siblings affected with early onset CAD and some CAD risk factors, suggesting a strong genetic component. A genome-wide linkage analysis was performed on this family using Illumina 370K Gene Chips. Linkage with a lod score of 1.8 to chromosome 19q13.11-q13.33 was observed. The interval spans 14Mbp and contains 446 annotated genes. *ApoE* was a promising candidate gene within the locus, but mutation screening of coding region of *ApoE* did not revealed a disease causing mutation. While attempting to minimize the region by studying other families, a few other promising candidate genes are being screened.

P08.16
TIMP2 polymorphism, left ventricular function and prognosis after myocardial infarction with ST elevation.

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Objective: Genetic variations of tissue inhibitors of matrix metalloproteinases (TIMP) are known to be involved in the process of development and progress of acute myocardial infarction. TIMPs play important role in process of plaque formation, destabilization and rupture within the myocard by regulation of extracellular matrix proteins degradation. In this study, we investigated the possible role of polymorphism in TIMP2 in acute myocardial infarction with ST elevations.
Methods: 556 patients with STEMI and 141 stable patients >50 years without MI in anamnesis and without LV systolic dysfunction were enrolled into the study. Levels of B-type natriuretic peptide (BNP) and N-terminal pro B-type natriuretic peptide (NT-proBNP) were measured at the time of hospital admission before primacy PCI and 24h, 3months and 12 months after MI onset. The TIMP2 intron polymorphism rs8176329 located at 17 chromosome was detected by RT-genotyping.
Results: No significant differences in genotype distribution and/or allele frequencies were observed when comparing STEMI patients and stable patients. Within the STEMI patient group lower prevalence of AA variant have been observed in subjects with acute heart failure (AHF) in time of hospitalization (p=0.048). We have found the association among GG genotype and higher BNP levels (p=0.03), higher NT-proBNP levels respectively
(p=0.008).
Conclusion: Taken together, our results provide evidence that GG variant correlate with higher levels of BNP and NT-proBNP, which strongly correspond to association of GG genotype with acute heart failure.

P08.17
Inference of cross-level interaction between genes and contextual factors in a matched case-control metabolic syndrome study: a Bayesian approach

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Background: Genes, environment, and the interaction between them are each known to play an important role in the risk for developing metabolic syndrome. Environmental influences can be measured at the individual level, such as by personal characteristics and lifestyle choices, or at the group-level (called contextual variables), such as by community factors and degree of local area development. To examine the interaction between genes and contextual factors, traditional approaches usually adopt asymptotic tests to assess whether the interaction exists. Such tests are limited as they cannot evaluate or quantify the strength of difference in genetic effects across different clusters or categories of the contextual variable. **Methods:** In this study, we utilize a Bayesian hierarchical mixed-effects model to investigate whether the genetic effect is modified by residential environment in a matched case-control metabolic syndrome study. The group-level contextual covariate, availability of exercise facilities, contains four categories, from low to high. Based on posterior samples from Markov chain Monte Carlo methods, the interaction between this group-level environmental condition and the individual-level genetic effect is evaluated by differences in allelic effects under various contextual categories. **Results:** The Bayesian analysis indicates that the effect of rs1801282 on metabolic syndrome development is modified by the contextual environmental factor such that, even with the same genotype, living in a residential area with low availability of exercise facility may result in higher risk. **Conclusions:** In summary, the Bayesian hierarchical mixed-effects model considered here provides a quantitative assessment for the cross-level interaction between genes and contextual variables.

P08.18

Time trends in the incidence of cystic fibrosis over a 40-year period

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Cystic fibrosis (CF) is a recessive disorder whose incidence has, for a long time, been estimated to 1/2500 live births in Caucasians. Newborn screening (NBS) programs allows now a better appreciation of the disease incidence that appears lower than in the past. Here we reported time trends in CF incidence over a long period (40 years: 1970-2009) in an area where CF is frequent (Brittany, France) and where NBS is implemented since a long time (1989).

The study enrolled the CF patients born in Brittany since 1970. The cases born before the set up of NBS were registered through active enquiries and combination of data sources. Time trends in incidence rates were examined using Poisson regression and expressed by the average percent change (APC).

Over the 40-year period, 569 CF children born in Brittany were registered. The average number of cases born each year varied from 17.7 in the 1970's (decade 1970-79) to 11.8 nowadays (decade 2000-09). This corresponded to a decline in incidence rate of 29.0% (from 1/2260 to 1/3183, p=0.0039). Poisson regression also showed that the incidence rate decreased over the whole period (annual APC: -1.2%, 95% CI: [-1.8; -0.5], p=0.0012), and especially since the availability of prenatal diagnosis in the 1980's (annual APC: -2.0%, 95% CI: [-3.7; -0.3], p=0.0216).

This study highlights how the incidence of CF evolved in an area where CF is frequent and where carrier screening is not underway. It reports a clear decline in incidence that results from a complex mixture of factors.

P08.19

The alpha2 isoform of the catalytic subunit of AMP variants have a key role to susceptibility to type 2 diabetes

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AMP-activated protein is a key regulator of cellular metabolism, and its activity is induced by both metformin and thiazolidinedione anti-diabetic medications. It has therefore been proposed both as a putative agent in the pathophysiology of type 2 diabetes (T2D) and as a valid target for therapeutic intervention. The gene encoding the alpha2 isoform of the catalytic subunit of AMPK (PRKAA2) is located at one of the Japanese T2D loci mapped previously. PRKAA2 is, therefore a good candidate gene for insulin resistance and T2D. We therefore set out to test for the association of common variants and common haplotype in the PRKAA2 gene with type 2 diabetes and related phenotypes. We undertook an extensive case-control association study using a total of 911 unrelated Japanese T2D patients and 876 control subjects at 6 single nucleotide polymorphism in the PRKAA2 gene. We observed association of nominal significance with two intronic SNP in the PRKAA2 (rs932447; OR=0.62; 95%CI=0.40-0.96; P=0.033, AND rs1418442; OR=0.62, 95% CI=0.40=0.96; P=0.030, both under a dominant model). However, we were unable to observe the association between the PRKAA2 haplotype and T2D. Our result indicate that the PRKAA2 gene variant have an important impact on T2D susceptibility in Japanese.

P08.20

Lack of association of the KCNJ11 E23K variant with risk of type 2 diabetes in a Iranian population

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The beta-cell ATP-sensitive potassium (KATP) channel consists of subunit of the inwardly rectifying potassium channel Kir6.2 and subunit of the sulfonylurea receptor 1 (SUR1).The gene KCNJ11 (Kir6.2) has a key role in glucose-stimulated insulin secretion and thus has always been considered as excellent susceptibility candidate for type 2 diabetes(T2D). An association between a common polymorphism in KCNJ11gene (E23K) and T2D has been reported in several but not all populations. We investigated whether E23K polymorphism in the KCNJ11gene is associated with T2D in the Iranian population. In this study, We performed a case-control association with 398 T2D patients and 420 Iranian control subjects using TaqMan assay. The case and control subjects had similar frequencies of the allele K (35.9 vs. 35.7%, P>0.05), then there was no significant difference between case and control subjects according to allele K frequency (OR =1.01, (95% CI) 0.825-1.236, P=0.928).Also, by genotype frequency comparison, no difference were observed between frequency of various genotypes regarding to diverse genotype models(ressecive , dominant and codominant model) of KCNJ11 E23K variant in our population. In conclusion, the presented study demonstrated the absence of the key role of KCNJ11 E23K variant in the occurrence of type 2 diabetes in a Iranian Population. Tis finding may conclusive due to limitation of sample size. A subsequent study with a larger sample size is recommended.

P08.21

The lipid-associated GALNT2 gene variant rs4846914 confers a highly elevated risk for type 2 diabetes

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The UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2 (GALNT2) has been recently implicated in the modulation of antgiopoietin-like protein - 3 (ANGPTL3) activity via O-glycosylation. GALNT2 is therefore indirectly contributing to plasma triglyceride level modifications as ANGPTL3 is an inhibitor of lipoprotein lipase, the enzyme responsible for triglyceride hydrolysis. Besides, recent genome-wide association studies identified GALNT2 variant rs4846914 in association with blood lipid level alterations. As the GWAS studies do not provide functional information, we studied and we supposed the possible association of this variant in type 2 diabetes mellitus (T2DM) and metabolic syndrome (MS) patients.

A total of 308 type 2 diabetic patients, 394 metabolic syndrome patients and 246 healthy controls were genotyped by PCR-RFLP method.

We detected a significantly higher ratio of GG homozygotes among both T2DM and MS patients than in controls. In addition, in multiple regression analysis adjusted for body mass index, triglyceride and total cholesterol levels as well as age and sex, the GG genotype is associated with the development of type 2 diabetes, with an unusually high odds ratio of 5.218 (CI [95%] 2.811-9.683; p<0.001), while carriers of the G allele demonstrate a lower risk of T2DM (OR 1.659; [CI95%] 1.027-2.678, p=0.038).

The data presented here revealed a differentiated risk nature of the lipid profile modifying GALNT2 natural gene variant rs4846914, as the variant did not show an association with metabolic syndrome in our cohort, but appears to confer a exceptionally risk for type 2 diabetes, which strong association that has not been reported in the literature available.

P08.22

Comparative genetics of gestational diabetes mellitus in humans and dogs.

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Gestational diabetes mellitus affects approximately 2-10% of human pregnancies and has a major impact on both maternal and foetal health. Family history of diabetes and ethnic background are two of the known risk factors for development of disease, which suggest a genetic background for the condition.

In dogs, the pregnant or the non-pregnant dioestral bitch develops peripheral insulin resistance, which can progress to diabetes mellitus. The dioestral phase of the canine sex hormone cycle mimics pregnancy

with regard to hormone levels and length. Elevated levels of growth hormone (GH) have been detected in diabetic dogs reflecting the placental GH secretion in human pregnancies. The dog has hence proven to be a good naturally occurring model of gestational diabetes. Epidemiologic studies show that certain dog breeds have a higher prevalence of diabetes suggesting a genetic predisposition as in humans.
We have performed genome-wide association studies in three high risk dog breeds: the Swedish Elkhound, the Norwegian Elkhound and the Border Collie, using the ~170k Illumina SNP array. Four preliminary loci have been identified, although more dogs are currently being genotyped. Candidate loci will be resequenced to identify candidate mutation and the functional consequences will be evaluated in dogs and humans. Candidate genes will also be examined in humans. We anticipate that our findings will progress the understanding of diabetes development in humans and dogs.

P08.23
A genome-wide association study in Doberman Pinschers identifies a new locus for dilated cardiomyopathy (DCM)

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Dilated cardiomyopathy (DCM) is a heterogeneous group of heart diseases with a strong genetic background. Currently, many human DCM cases exist, where no causative mutation can be identified. DCM also occurs with high prevalence in several large dog breeds. In the Doberman Pinscher a specific DCM form characterized by arrhythmias and/or echocardiographic changes has been intensively studied by veterinary cardiologists. We performed a genome-wide association study in Doberman Pinschers in the frame of the EU-LUPA project. Using 71 DCM cases and 70 controls collected in Germany we identified a genome-wide significant association to DCM on dog chromosome 5. We validated the association in an independent cohort collected in the United Kingdom. The raw p-value of the best associated SNP in the combined cohorts is 7.04×10^{-10}. There is no known DCM candidate gene under the association signal. Therefore, DCM in Doberman Pinschers offers the chance of identifying a novel DCM gene that might also be relevant for human health.

P08.24***
Relation between DNA methylation and gene expression

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Epigenetic mechanisms are important for regulating the cellular machinery including gene expression. DNA methylation is one form of epigenetic control that is most commonly studied. However, little is known about the complex relation between DNA methylation and gene expression. This study is an effort to decipher how DNA methylation affects gene expression.
For this study we used (Illumina) array-based genome-wide DNA methylation and gene expression data obtained in whole blood of 150 healthy controls. After quality control and removal of batch effects we performed regression analysis with DNA methylation as a predictor of gene expression levels. Age and gender were used as covariates. We tested association of every methylation probe with every gene expression probe, resulting in over 400 million tests. Significance thresholds were adjusted for *cis* (region of 500 kb of both sides of a gene) and *trans* (genome-wide). Available genome-wide SNP data was used to investigate the genetic control of DNA methylation and gene expression at these loci.
We observed both positively and negatively associations between gene expression probes and DNA methylation probes. For *cis* 3,615 combinations of 386,993 possible combinations were significant, of which 1,932 negatively and 1,683 positively (53.4% vs 46.6%). Explained variance from the DNA methylation levels ranged from 1 to 52 percent. Overall the results show that DNA methylation levels and gene expression levels are both positive as well as negatively correlated. Data on CpG properties, genetic regulation and *trans* association provide further insight into the complex processes of gene expression and DNA methylation.

P08.25
Identifying genetic determinants of congenital heart defects in Down syndrome

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Congenital heart defect (CHD) occurs in ~40%, and AVSD (Atrio-Ventricular Septal Defect) is observed in 16% of Down syndrome (DS) cases. Here, we test the hypothesis that genomic variation in concert with the trisomy 21 determines the risk for CHD. This case-control GWAS includes 156 DS without CHD and 192 DS with CHD (AVSD=72, ASD=53, VSD=67). Trisomic and disomic SNPs were called separately. Trisomic single-locus association reveals 4 SNPs as CHD risk alleles (Bonferroni adjusted p-values < 0.05). One of these SNPs was also identified as a risk factor for AVSD+VSD combined (p=0.008), while a 5th SNP was identified as risk factor for ASD (p= 0.002) and AVSD+ASD combined (p=0.05). No significant signal was identified from the non-chr21 GWAS. We then performed a 2-locus genomewide analysis. A significant interaction signal was observed between loci on chr5 and chr6 (p=0.002). Since DS is likely to be a disorder of gene expression, we focused on 2-locus interactions using only eQTLs. Interestingly, 2-locus model analysis on chr21 eQTLs reveals an interaction between eQTLs for PDE9A and WDR9 genes and eQTLs for C21orf56 and C21orf57 genes.
Furthermore, the 2-locus analysis of non-chr21 eQTLs identifies an interaction between eQTLs for CLCNKA on chr1 and USMG5 on chr10 (p=0.01). A search for chr21 risk CNVs for AVSD using tiling 135K chr21 array did not show any significant risk variation. We propose that the CHD risk of DS is determined by not only trisomy 21 but also the genome-wide interaction of specific alleles.

P08.26
A novel locus for autosomal recessive dystonia with mental retardation maps to chromosome 6

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Introduction: Dystonia is a clinical syndrome with sustained muscle contraction, twisting, and abnormal postures. Some cases are familial, but most are sporadic and of uncertain cause. Presently, ten genes and seven additional loci have been reported to be associated with monogenic primary dystonias. We aimed to identify the genetic cause of dystonia in an affected Iranian family.
Materials & methods: Whole genome homozygosity mapping was performed in a consanguineous Iranian family with three dystonia affected children using high density single nucleotide polymorphism chips**.** Affected individuals were DYT1 negative and exhibited childhood-onset, segmental dystonia and mental retardation.
***Results*:** Haplotype analysis showed that all the affected individuals shared a common haplotype expanding 13cM on chromosome 6. Parametric linkage analyses resulted in a lod score of 1.6. More than 200 annotated genes exist within the linked region. Screening of four candidate genes has not revealed a disease causing mutation.
Conclusions: These findings indicate that a novel dystonia gene exists on chromosome 6. Mutation screening of other candidate genes within the linked region is being performed.

P08.27

Genetic of EBA

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Epidermolysis bullosa acquisita (EBA) is an autoimmune blistering disease, characterized by antibodies to type VII collagen (COL7). At the moment little is known about the genetic susceptibility for EBA. To find key genetic factors (MHC and non-MHC EBA susceptibility genes) that control inflammation at the effector phase of EBA, we used a multi-step approach. First, different and 16 genetically well characterized inbred strains were tested for their susceptibility for EBA in the passive model. This allowed the identification of 8 associated loci indicating EBA severity to be regulated by loci on chromosomes 1, 6, 8, 9, 11, 12, 15 and 16. To confirm these, we used mice from an advanced 4-way intercross breeding of 4 different strains with different major histocompatibility complex (MHC) haplotype (MRL, NZM, BXD2, and Cast). For example on chromosome 8 and 9 that have already been tested, we could confirm the genes on chr 9. We demonstrated that low statistical significant of full Genome-Wide Association Study with characterized inbred mouse strains together with outbred family of mice allows for individual gene discovery for classic genetic linkage analysis.

P08.28

Whole genome linkage analysis in a large consanguineous Turkish family with idiopathic generalized epilepsy

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Epilepsy is a complex neurological disorder that affects 1% of the world's population. Among different forms of epilepsies, idiopathic generalized epilepsies (IGE) are characterized by bilateral and synchronous generalized seizures in the absence of detectable brain lesions or metabolic abnormalities. Thus, the primary etiology for this disorder is believed to be genetic.

The proposed study includes a large highly inbred consanguineous family with multiple IGE affected individuals. The ultimate aim is identifying a new epilepsy gene to help in delineating the molecular mechanisms of this disease. Physical, neurological and EEG examinations were performed on the subjects recruited with information on family history. These examinations revealed 5 affected individuals, a mother with three children and their cousin, who exhibit varying types of seizures. Along with these individuals, 4 unaffected individuals from this family were genotyped using Illumina Infinium HumanLinkage-12 Genotyping BeadChip (6090 SNPs). The genotype data were delineated using easyLinkage software platform, where Mendelian genotyping errors were determined using PedCheck, two and multipoint lod scores were calculated under the assumption of autosomal recessive inheritance via SuperLink and SimWalk, respectively, and haplotypes were constructed through GeneHunter. Analyses revealed a promising locus on chromosome 2p12, which was further analyzed through fine-mapping with additional microsatellites spanning a region of 75.36Mb-78.52Mb. However, microsattellite alleles were heterozygous in the 3 affected sibs rejecting linkage under autosomal recessive inheritance.

Future objectives include acquisition of extensive clinical data and dense SNP genotyping of the family members in an attempt to unravel small regions that may have been overlooked.

P08.29

The eNOS 4a/5b polymorphism increases the risk for end-stage renal disease in Romanian diabetic patients

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Background: Diabetic patients have a high risk of developing end-stage renal disease (ESRD). Some polymorphisms seem to increase the risk for renal failure in different populations.

Objectives: The aims of this case-control study were to estimate the possible impact of eNOS 4a/5b, MTHFR C677T, MTR A2756G, ACE-ID, ATR1 A1166C, HSPG2 BamH1 and TGFb C/T polymorphisms on the ESRD development in diabetic and nondiabetic patients.

Materials and methods: Clinical information and blood samples were collected from 1350 unrelated Caucasians represented by dDM1 (type1 diabetes mellitus dialysed patients, n=225), DM1 (type1 diabetes mellitus patients without renal disease, n=180), dDM2 (type 2 diabetes mellitus dialysed patients, n=225), DM2 (type 2 diabetes mellitus patients without renal disease, n=180) and HC (healthy controls, n= 540). Genomic DNA was extracted from blood samples with Wizard Genomic DNA purification Kit (Promega). Genotyping was performed by PCR and RFLP techniques.

Results: The frequencies for these genotypes are in Hardy-Weinberg equilibrium. Our results are not in agreement with several earlier studies which have indicated a strong association between investigated polymorphisms and ESRD in some Caucasian populations. Only eNOS 4a/5b increases the risk for renal failure in DM1 and DM2 patients (OR=2.2).

Conclusion: Our study, performed in Romanian population, shows that eNOS 4a/5b polymorphism is associated with ESRD development in diabetic subjects.

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P08.30

EX-HOM: EXome HOMozygosity in small consanguineous pedigrees

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Objective: We provide the proof of principle that exome sequencing of only two affected siblings born to first cousin parents is capable of directly identifying a single candidate gene for an autosomal recessive disorder. This strategy, which we call EX-HOM (EXome-HOMozygosity), combines in a single step the capacity of exome sequencing to identify all the coding variants present in a genome with the property of homozygosity mapping to limit the search for candidate genes to specific chromosomal regions.

Methods: We sequenced the exomes of two siblings born to first cousin parents affected with dysmyelinating leukodystrophy and spastic paraparesis caused by a mutation in *FA2H* . We extracted homozygosity information directly from exome sequencing data, without performing previous SNP genotyping, and selected the candidate variants contained in the homozygous regions shared by the two affected siblings.

Results: We identified five regions of shared homozygosity, accounting for 94.2 Mb, and containing three candidate variants. Among these, the known *FA2H* mutation remained the only prominent one.

Conclusion: In small consanguineous pedigrees, the EX-HOM approach is a direct way to identify the candidate genetic defect, bypassing obstacles such as genetic heterogeneity and the need for large pedigrees.

P08.31

Determining R202Q allele frequency in FMF patients

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Familial Mediterranean Fever (FMF) is an autosomal recessive disease characterized by recurrent attacks of serositis, abdominal pain, arthritis and pleuritis. FMF frequenty affects populations around the eastern Mediterranean origin, especially Sephardic Jews, Armenians, Turks, Arabs and Greeks. Disease is caused by mutations in MEFV gene, which encodes a protein named Pyrin/Marenostrin. In the Infever Database, more than 180 gene alterations polymorphisms/mutations located in the MEFV gene.

In the present study, we used 100 FMF patients and 104 healthy controls. We detected R202Q mutation (605 G>A) at exon 2 of the MEFV gene in Turkish FMF patients and healthy controls.
We tested the existence of the mutation by PvuII restriction enzyme digestion after PCR amplification of the exon-2. In patients group, we detected homozygous R202Q mutation (AA allele) in 9 people, and heterozygous R202Q mutations in 49 subjects. However, in control group, we detected homozygous R202Q mutations in 3 subjects and R202Q heterozygous mutations in 27 subjects.
The present study demonstrats that R202Q allele could be a risk factor for FMF. Currently, we are testing a higher number of subjects in both patient and control groups.

P08.32
The effect of missing data on type I error rate of family-based association tests in the presence of linkage and no association

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One common element in family-based association studies is incomplete data, meaning missing phenotype and/or genotype information, especially on late onset diseases. There are several family-based association analysis methods which claim that missing data is not an issue and proper type I error rates are holding.
In this study, we examined the type I error rates of several commonly used family-based association tests in genetic epidemiology as to their performance under increasing proportion of missing data on parents and/or founders using mixtures of singletons and families. We assumed complete linkage, but no association between disease and marker loci under a variety of inheritance models.
The results of our study showed enormous type I error rate with some popular methods when proportion of missing data was increasing. This can lead to false conclusions of positive association and therefore wasting of resources in follow up studies. Results from a simulation-based study will be presented.

P08.33
Lack of association between single nucleotide Polymorphism and BCL11A HBS1L-MYB situations with increased levels of fetal hemoglobin

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Variable levels of fetal hemoglobin may persist into adulthood, although they have no clinical consequences in otherwise healthy people. High HbF levels have a major impact on the hemoglobin disorder such as Beta- Thalassemia Major. Increased HbF production ameliorates the severity of both diseases. The level of HbF in adults is inherited as a quantitative trait, and is largely genetically controlled with a heritability of 89%. HBS1L-MYB intergenic regions on chromosome 6q23, BCL11A on chromosome 2p16 and γ-globin genes on chromosome 11, account for up to 50% of the variation in HbF levels in patients with sickle cell anemia or Beta- Thalassemia Major thalassemia and in healthy adults. Common single nucleotide polymorphisms (SNPs) at the BCL11A and HBS1L-MYB loci have been implicated previously in HbF level variation. In this study, three common SNPs were genotyped for rs4895441, rs11886868 and rs28384513 using PCR-RFLP method among 50 major β-thalassemia patients with high levels of HbF. Digestion carried out by RsaI, MboII and BstxI respectively. Mutant allelic frequencies were in healthy patient. P-value was higher than 0.05 at three SNPs. There was no association between the studied polymorphisms and the variation in HbF levels in patients. Other variations may have a role in enhancing the fetal hemoglobin in our population. Different populations have different variants modifying the severity of a sickle cell anemia and Beta- Thalassemia Major in the word.

P08.34
Study of polymorphic variant of gene alleles of apolipoprotein C-3 (APOC-3) in individuals with different levels of high density lipoprotein

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Genetic factors, along with environmental play an important role in the determination of the lipid metabolism in humans. APOC-3 gene (11q23.1-q23.2) encodes a protein apolipoprotein C-3, which is one of the main regulators of lipid metabolism in the human body and, in particular, regulates intravascular triglyceride splitting. We studied polymorphic gene variant C3238G APOC-3 and analyzed associations of the polymorphic variant with a level of high density lipoprotein (HDL) of human serum.
Material for the study included DNA samples from 339 individuals. Determining the level of HDL cholesterol by standard enzymatic methods. Analysis of polymorphic variant APOC-3 (C3238G) was performed by PCR-RFLP.
Results: The group of persons with low levels of HDL cholesterol in the blood serum revealed increase of genotype APOC-3 *G/*G (83,33% vs. 74% in the group of individuals with indicators of HDL in normal, P = 0.0005). When comparing those with high levels of HDL (79.1%) and those with rates of HDL do not normally found statistically significant differences.
Conclusions: The found that the polymorphic variant C3238G gene APOC-3 is associated with the level of high density lipoprotein (HDL), and genotype APOC-3 *G/*G is a marker of low levels of HDL.

P08.35
Detecting gene effect by simultaneously testing many minuscular gene-gene or gene-environment interactions

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We proposed a new approach for detecting the gene effect by simultaneously testing many small gene-gene (GG) or gene-environment interactions. The presence of GG interactions indicated that the gene is susceptible to the outcome, in spite of the state of the marginal effect. The interaction effects between two genes were determined by an interaction term in a logistic regression model. Test statistic is summation of Wald chi-square values of GG interaction tests in the regression models. If each Wald test for interaction is independent, under null hypothesis, the test statistic followed a chi-square distribution with m (number of GG interaction tests) degrees of freedom. If Wald tests are not independent, significance for test statistic is evaluated by permutation. For this approach, power evaluation in four scenarios according to asymptotic power functions determined by sample size and number of genes interacting with the gene of interest. Requirement for binary variables and being unfavorable for rare gene are major limitations of this approach.

P08.36
A genome-wide genetic map based on the ERF study data

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In multipoint linkage studies, the Lod-score values can depend much on the genetic map in use. It is desirable for a genetic map to reflect the true frequencies of recombination events in studied population, at both overall scale and local level.
In this study, a genome-wide genetic map was built using CRIMAP software on a sample of 2834 members of a large pedigree from a young isolate in the Netherlands genotyped for 5549 SNP markers of Illumina 6k linkage panel as a part of the ERF study. The large pedigree was split into 391 smaller subpedigrees with no overlapping in meioses. The total sample included 1928 meioses with mean of 716.2 and 1287.8 informative meioses per SNP marker on autosomes and X chromosome, respectively.
The total genetic length of sex-averaged, male and female ERF genetic map were 3288.5, 2325.1 and 4094.6 cM, respectively, which

is ~12.8% shorter than the sum of corresponding intervals of Rutgers map v.2, the finest genetic map available to date for Caucasians. At the local level, 73 between-marker intervals showed the strongest differences in genetic length between two maps (the Rutgers estimate fell out of 99.99% CI of the respective ERF map interval). Unexpectedly, 46 (63%) of these intervals were longer for ERF map despite its shorter total genetic length. As all these differences can reflect the true features of local and global recombination rates in ERF population, the information obtained can be used to refine genetic maps for linkage analysis of ERF data.

P08.37
Dealing with stratification and cryptic relatedness in GWAS: a case study in a canine population.

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Canine models are constantly gaining importance in biomedical studies and have been successfully used to increase our understanding of diseases in human. However, canine populations often show significant degree of stratification and cryptic relatedness. Several methods have been proposed to account for genetic structure: genomic control, structured association, PCA-based and mixed models.

The majority of the methods have been designed to be used primarily for the analysis of human populations and to account for only some aspects of genetic structure. Therefore, genome-wide association studies in canine populations often call for more specific solutions. Here, we present a workflow that we successfully applied to analyze a structured canine population with cryptic relatedness present.

We analyzed 184 German shepherds (91 cases and 93 controls) using Illumina 170K canine SNP array. First, we used autosomal IBS matrix, MDS and K-means clustering to determine population structure. We confirmed that apart from population stratification, there is some degree of cryptic relatedness between the individuals. Next, we tested 4 different methods of dealing with genetic structure: 1) structured association (SA), 2) PCA-based approach (PCA) 3), mixed model (MM) and 4) a combination between mixed model and structured association (MM-SA). We obtained the following values of the λ inflation factor (λ>1 indicates that the association test statistics is inflated due to genetic structure): λ_{NO_GC}=1.93, λ_{SA}=1.15, λ_{PCA}=1.07, λ_{MM}=1.00 and $\lambda_{MM\text{-}SA}$=1.02.

We found the MM and MM-SA approaches to be most suitable to account for both the population stratification and cryptic relatedness.

P08.38
Comparison of two strategies for genotype calling

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Sample size needed to perform Genome-wide Association scans is drastically increasing. Recent studies have shown that batch effects are not negligible when clustering algorithms are used to perform genotype calling (GC); thus calling becomes a challenging step in large data sets. To reduce the run time while minimizing batch effects we evaluated two different strategies to call genotypes on a distributed computing environment, using a cohort of > 6,000 individuals genotyped with the Affymetrix 6.0 array, and the Birdseed-v2 calling algorithm included on the Affymetrix Power Tools (APT) package. First, we divided the probesets in batches of 50,000 probes with 5,000 overlapping and ran jobs in parallel. Since the same normalization matrix was used by all jobs, the results are expected to be equivalent to those obtained in a unique run. GC was completed on 15 days, using 18 CPU with 16Gb RAM, but required large amount of disk space. To further reduce the run time, we ran the APT on 7 clusters of 1,000 individuals with 100 overlapping, balancing the ratio of cases and controls to mimic the proportion observed in the entire dataset, and maximizing plates representation within each group. GC was completed in 2 days, using only 6 CPU and significantly less disk space. Discordance rate was 0.1% between genotypes of overlapping individuals among clusters, and on average, 0.45% of genotypes differed from those called with the first strategy. Thus the second approach can be a valid alternative for conducting GC in large data sets.

P08.39
Rules for resolving Mendelian inconsistencies in nuclear pedigrees typed for two-allele markers

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Gene-mapping studies regularly rely on examination for Mendelian transmission of marker alleles in a pedigree, as a way of screening for genotyping errors and mutations. For analysis of family data sets, it is usually necessary to resolve or remove the genotyping errors prior to analysis. At the Center of Inherited Disease Research (CIDR), to deal with their large-scale data flow, they formalized their data cleaning approach in a set of rules based on PedCheck output. We examine via carefully designed simulations that how well CIDR's data cleaning rules work in practice. We found that genotype errors in siblings are detected more often than in parents for less polymorphic SNPs and vice versa for more polymorphic SNPs. Through computer simulation, we conclude that some of the CIDR's rules work poorly in some situations and we suggest a set of modified data cleaning rules that may work better than CIDR's rules.

P08.40
New glaucoma related locus on chromosome 20p13

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Morphologic characteristics of the optic disc are associated with primary open angle glaucoma and can be considered as its endophenotypes. To identify new genes related to glaucoma we analyzed the endophenotypes in large pedigree from Dutch isolated population. We performed genome-wide linkage and association analyses of combined quantitative traits obtained by principal component analysis of seven parameters of the optic disc (disc area, rim area, superotemporal and inferotemporal rim areas, rim-to-disc area ratio, cup-to-disc ratio and cup shape measure) measured in 1,979 participants. Two independent principal components and two factors were generated. The first principal component, PC1, explained about 52% of the total variance and had the high loadings of four rim related traits and cup-to-disc ratio. In the factor score FAC1 obtained by rotation of PC1, the number of initial parameters with high loading was minimized to three rim related traits. We found the strongest linkage signal (LOD = 4.46) for FAC1 on chromosome region 20p13. This signal has been confirmed by suggestive linkage peak for PC1 (LOD = 2.28) and association of rs58532 in the linked region with PC1 (the lowest *p*-value = 8.2×10^{-6}, FDR *q*-value = 0.027). The associated region points to a cluster of genes encoding several members of signal-regulatory-protein (SIRP) family, the regulatory membrane proteins. The closest gene, *SIRPA*, is likely to be involved in IGF-1-mediated AKT-activation, which has an anti-apoptotic effect. The new locus suggests a link between growth regulation and rim related parameters that are endophenotypes for glaucoma.

P08.41
Haplogrep as a tool for mitochondrial DNA analysis

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In the last decades, the analysis of variations in mitochondrial DNA in human populations has provided a lot of information about things like molecular anthropology, genealogy and some genetic diseases. Thanks to web programs like HaploGrep, based on the data of Phylothree.org and updated at the same time that Phylothree.org database, we could manage a big amount of information about sequences of DNAmt. These kind of programs makes it easier and quicker the processing of all the data and obtaining a classification of a hundred of samples into different

haplogroups. HaploGrep also informs us about the probability of each sample in the assigned haplogroup, the probability of the followed haplogroups with a high value and the most representatives' mutations of each single haplogroup. The obtained results are represented in a graphic way like tables and phylogenetic threes. In this study we could observe different applications of this web program. One is to see the genetic influence in the population flow of an ancient population (North African) in the actual South Spanish population. Another is the analysis of the crossbreeding in South American populations as a result of Spanish and Mayan admixture. The last one consists in the use of this program analyzing the similarities and differences between patients and controls in diseases like cancer. Whereas in crossbreeding and population studies the utility of this program has been proved, we are testing how useful it could be in the analysis of cancer although some clinical significative differences have been marked.

P08.42**

A genome-wide association study in European isolated populations identifies new loci and pathways for hearing function, thresholds and age-related loss

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Hearing is a complex trait but, until now, few genes are known to contribute to its variability and little is known about genetic factors involved in age-related hearing loss (ARHL). To discover genes and pathways underlying auditory function and ARHL we planned an integrated strategy characterized by three approaches. A) a GWAS meta-analyzing quantitative traits data from 6 isolated populations . Results led to the identification of eight suggestive significant loci ($p<10^{-7}$) with a series of genes expressed within the inner ear. Additional biological candidates ($p<10^{-6}$) were identified. Some of these new loci map to already known hereditary hearing loss loci whose genes still need to be identified. Data have also been used to construct a highly significant "in silico" pathway for hearing function characterized by a network of 49 genes, 34 of which are certainly expressed in the ear. B) a similar GWAS on qualitative traits from the same populations leading to the identification of some loci on the following chromosomes 2,13,19,17,16,20 involving in some cases genes related with hearing development and hearing function but also genes whose function is still unknown. A replica phase is now in progress on a series of case/controls coming from different European countries. C) whole exome sequencing of cases selected from large pedigrees showing segregation of ARHL and coming from the same isolated populations. Preliminary results provide new insights into the molecular basis of hearing function and ARHL and may suggest new targets for hearing impairment treatment and prevention.

P08.43

Genetic Analysis of Autosomal Dominant and Recessive Hereditary Spastic Paraplegia

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Hereditary spastic paraplegias (HSPs) describe a heterogeneous group of neurodegenerative disorders with progressive spasticity in the lower limbs. HSPs can be inherited in autosomal dominant, recessive, or X-linked forms. To date, 41 spastic paraplegia loci and 17 genes have been identified with diverse functions such as axonal transport, regulation of the cytoskeleton, and mitochondrial function.
In the first part of the study, the most commonly mutated autosomal dominant genes, SPG4 (Spastin), SPG3A (Atlastin), SPG31 (REEP1), ve SPG7 (NIPA1) were screened in 42 families. Using the SSCP method and subsequent DNA sequencing one patient and his father were found to have an insertion mutation (c.310_311insA) leading to a truncated Spastin protein. Another patient revealed a heterozygous c.1741C>T change (p.Arg581X) leading to a premature stop codon in the same gene. Still one other patient was heterozygous for a missense mutation, c.941A>G (p.H258R) in *Atlastin.* All identified mutations were previously reported.
In the second part of the study, linkage to autosomal recessive loci for which the genes have been identified (SPG5, SPG7, SPG11, SPG15, SPG20 and SPG21 loci) was investigated in 15 families. Parents were consanguineous in 11 families with at least two affected siblings. Linkage was found to SPG11 in four families, to SPG15 in two families, and to SPG21 in one family. Sequencing of the relevant genes is currently being performed to identify the causative mutation in these families. In two of the families, linkage to all tested loci was excluded that should be further investigated for other known AR-HSP loci.

P08.44

The HIF1A Pro582Ser polymorphism in Russian weightlifters

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Power performance is determined by high anaerobic potential (high reserves of ATP, creatine phosphate, and glycogen; high concentration and activities of glycolysis and phosphagenic system enzymes; predominance of fast-twitch muscle fibers in skeletal muscles, etc.). *HIF-1* gene, expressed in all mammalian tissues, attends in organism's response to hypoxia via controlling glucose metabolism and genes involved in different cellular functions. HIF1A, the regulatory subunit of *HIF-1* gene's dimeric product, contains a missense polymorphism Pro582Ser (C/T at bp 85; rs11549465). The rare T allele (proline to serine change) by increasing HIF-1A protein's activity and stability may improve glucose metabolism. The study aimed to determine the influence of the *HIF1A* Pro582Ser polymorphism on power performance. One hundred and eight professional weightlifters of regional and national standard were recruited for the study. Controls were 434 healthy unrelated volunteers, citizens of Kazan, without any competitive sport experience. Genotyping was performed on DNA samples obtained from buccal epithelium cells by DNA extraction kit. Genotyping for the *HIF1A* Pro582Ser variants (rs11549465) was performed by PCR and restriction enzyme digestion. The number of carriers of the rare Ser allele (Pro/Ser or Ser/Ser) was significantly higher in weightlifters than in controls (28.7% vs. 13.4; P=0.0001). Thus, *HIF1A* gene Pro582Ser polymorphism is associated with elite power athlete status, which suggests an important role for HIF-1a in skeletal muscle adaptation to power training.

P08.45

Identification Of The Gene Responsible For The 4H Syndrome.

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Dentoleukoencephalopathies with autosomal recessive inheritance are very rare. In 2003, we reported, for the first time, a large inbred Syrian pedigree with oligodontia associated with a degenerative neurologic condition characterized by progressive ataxia, pyramidal syndrome, central white matter abnormalities and cortical atrophy (AJMG, 2003,118A(1):76-81).
A SNP genomewide linkage analysis, in this family, led to the identification of a candidate linkage region at chromosome 10q22, with a maximum multipoint lod score value of 5.66 (NPL score = 7.65). The candidate genomic interval contained 95 known genes including the Prosaposin gene (*PSAP)* responsible for metachromatic leukodystrophy, which was excluded. Sixteen additional candidate genes were screened by fluorescent sequencing and excluded.
Sequencing of the seventeenth candidate gene revealed an intronic variation which segregates perfectly in the family and affects the

splicing of the corresponding transcript.
Screening of this gene was performed in 5 supplementary families affected with the 4H syndrome (Leukodystrophy Hypomyelinating with Hypodontia and Hypogonadotropic Hypogonadism), a condition similar to the one we described in the Syrian family, but with additional hypogonadotropic hypogonadism. Several different missense and nonsense point mutations were detected in the gene, therefore demonstrating the implication of this gene in both the 4H syndrome and the syndrome described by us in the Syrian family.
Functional analyses are underway in order to understand the physiopathology of these syndromes. Results will be published soon.

P08.46
Fast Linkage calculation of Affymetrix SNP 6.0 genotype data using a new program SNP6-LINK

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Ott 1976 (Am J Hum Genet 28, 528-529) introduced the program LIPED for linkage analysis of large family pedigrees with or without loops and the program is ideal for two-point linkage analyses. We have previously constructed a helper program, LIPED.COM, that automatically make an input file to LIPED, allowing LOD score calculations for more than 800 markers and the results to be present in a usable form. This program has been tested by mapping more than 800 markers using families from the Copenhagen Family Bank (Eiberg et al. Clin Genet 1989; 36:415-418).
The development of SNP microarray chip technology (Affymetrix chip) for testing >10^6 SNP alleles has made new demands for the linkage analysis. We have therefore modified our linkage helper programs to analyze genotype output files for > 10^6 loci. The materials can be analyzed fast and the results shown graphically. This program, SNP6-LINK, demands a simple FORTRAN programming for each new project and several families can be analyzed in one process and the program can use Illumina genotype output files. Several parameters can be adjusted before calculations as heterozygote mating for recessive traits, which reduce the output graphical presentation to fewer but informative positive or negative LOD scores. Also bed files for uploading of LOD scores to UCSC Genome Browser can be produced. The LOD score calculations for more than a million SNP's in nuclear families takes less than half an hour, and can be run using low RAM PC with Windows Excell and a FORTRAN 99 compiler.

P08.47
A candidate gene study for Major Depressive Disorder in STAR*D and GAIN cohorts identifies potential statistical interaction between several candidate genes.

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Major depressive disorder (MDD) is a severe lifelong psychiatric illness with a heterogeneous background likely caused by interaction of a multiple set of genetic and environmental influences. Despite decades of genetic studies no genetic risk factor has been successfully validated for MDD.
Most genetic studies have so far relied on single SNP association analyses. However, it is likely that in the susceptibility of multifactorial diseases risk factors interact to increase disease susceptibility. In the present study we have analyzed interactions of SNPs in the GAIN MDD-study located in candidate genes selected from the STAR*D-cohort.
Forty-seven markers that were found to be nominally associated in the GAIN MDD-study were included in the study. Each marker was then tested for interaction with 2866 genotyped or imputed markers in the same set of genes. The interaction model assumed a dominant effect from one marker. Interaction was then defined as deviation from additivity and measured using the attributable proportion. Odds ratios (OR's) were used as risk estimates. Departure from additivity indicated that two factors were jointly involved in disease susceptibility. We identified a total of 16 interactions. The strongest p-values were in the order of 10(-14) and represented interactions between markers in GRIK4-NR3C2, ARHGAP10-GRIA2 and HTR2C-FKBP5 genes.
A subsequent pFDR-test confirmed that the interaction effects were robustly reported. However, using OR's may have inflated the risk estimates. Therefore, these results are currently validated using a permutation-analysis.

P08.48
A detailed view on Model-Based Multifactor Dimensionality Reduction with quantitative traits for detecting gene-gene interactions: different ways of adjusting for lower-order effects

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Understanding the effects of genes on the development of complex diseases and traits in humans is a major aim of genetic epidemiology. In particular, if there exist significant main effects unaccounted for during epistasis screening, false positives arise and therefore epistasis results reported might not be genuine. In this study, we use simulation to assess the effect of implementing different corrective measures for lower-order effects in an MB-MDR context of two-locus detection, on both power and family-wise error rate.
The implemented MB-MDR strategy reduces the multi-locus genotype dimension to one with three possible outcomes, while pooling multi-locus genotypes into three groups of risk (High, Low and No Evidence) based on the liberal p-value threshold 0.1. Adjusting for main effects is performed in two categories: main effects screening prior to and during MB-MDR run.
Data are simulated according to two quantitative trait models (M27 and M170) of Evans et al. 2006 that incorporate varying degrees of epistasis, and include a range of allele frequencies p: 0.1, 0.25, 0.5, at the predetermined functional SNPs. We consider 500 simulation replicates, involving 2000 unrelated individuals. MB-MDR screening is performed on 100 SNPs which are assumed to be in Hardy-Weinberg Equilibrium. No linkage disequilibrium exists between the SNPs.
In conclusion, preliminary results showed that indeed uncorrected for main effects results have false positives amounting to as high as 100%. Also, additive modeling, the mainly used first strategy in the context of main effects GWAS may not be sufficient for epistasis screening.

P08.49
Imputation-free Meta-Analysis with YAMAS (Yet Another Meta-Analysis Software)

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The success of GWAS is motivation enough to exchange and combine available data resources to expediently discover genetic risk factors for human traits. The primary tool is meta-analysis (MA) of imputed data.
A main purpose of imputation prior to MA is to avoid loss of SNPs. With YAMAS we present a software that avoids such loss without the need to impute. By using reference data from the HAPMAP and 1000 genomes projects users are enabled to analyze all SNPs that are present in at least one study. For each SNP that is missing in one study, the marker from the study with largest r^2, according to the reference data, with the missing marker is chosen as a "proxy SNP". Furthermore, based on reference haplotype frequencies, "proxy alleles" of a SNP and its proxy-SNP are identified. For each SNP, MA of that SNP is done by combining the association results of the SNP or, if not available the proxy-SNP, across studies.
We present results from a power simulation study and compare the performance of MA with imputation and imputation-free MA. Imputation-free MA in general is not quite as powerful as MA with imputation, since the marker panel is still smaller. However, in most scenarios, the power loss is only very moderate.
In summary, MA with the YAMAS proxy-algorithm is a quick and easy

alternative, yielding ad hoc results and thereby giving an incentive to follow-up analysis. Keeping in mind that collaborative MA efforts are frequently long-lasting, the time advantage becomes apparent.

P08.50
MicroRNAs 340* AND 624* are upregulated in platelets in patients with coronary artery disease

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Rationale Coronary artery disease (CAD) is a cause of mortality, underscoring the need for innovative diagnostic strategies. Platelets play a role not only in acute thrombotic disease, but also in the process of atherosclerotic plaque formation. This is exemplified by the beneficial use of anti-platelet therapy. MiRNAs are emerging as attractive biomarkers since they exhibit tissue- and cell-specific expression. We aim to identify novel biomarkers for diagnostic strategies. We hypothesized that platelet derived miRNA expression patterns differ between patients with CAD and controls.

Methods & Results We isolated RNA from platelets of 12 male patients with premature CAD and 12 age- and sex-matched healthy controls and performed miRNA array expression profiling (Illumina beadchips). We validated our findings in two independent cohorts by qPCR. Microarray profiling identified 214 of the 893 mature human miRNAs to be differentially expressed ($p< 0.05$). When corrected for background noise and corrected for multiple testing, miR340*, miR451, miR454*, miR545:9.1, miR615-5p, and miR624* and miR1280* were identified with an expression level that was at least 1.5-fold different in patients as compared to controls. The array and validation demonstrated that miR340* and miR624* had significantly higher expression levels in patients as compared to controls ($P< 0.05$).

Conclusion Two platelet derived microRNAs are significantly upregulated in patients with CAD as compared to healthy controls

Clinical Relevance Platelet MiR340* and miR624* are upregulated in CAD, therefore they may be a potential biomarker in CAD.

P08.51
Investigation of five loci (DFNB6, DFNB25, DFNB39, DFNB42, DFNB91) in 100 Iranian families with Autosomal recessive non-syndromic hearing loss by linkage analysis

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Hearing loss in humans is the most genetically heterogeneous trait known. This impairment affects more than 270 million people worldwide. Genetic causes of hearing loss are estimated to account for 68% of cases in newborns and 55% of cases by the age of four. Autosomal recessive non-syndromic hearing loss (ARNSHL) is the most common type of inherited hearing impairment. Due to the wealthy gene pool, Iran is a valuable source to identify the genes involved in different conditions.

To date, several genes have been studied in deaf Iranian population. Here we are studying 5 genes (*TMIE*, *GRXCR1*, *HGF*, *ILDR1*, and *SERPINB6*) for mutations that result in ARNSHL. Mutations in these genes have been identified in Iranian, Indian, Pakistani and Turkish families.

The objective of this study was to determine the prevalence of mutations in these genes in the Iranian population. A total of 100 families with ARNSHL are being investigated by screening for homozygosity to these five loci (DFNB6, DFNB25, DFNB39, DFNB42, and DFNB91). After homozygosity mapping is completed, sequencing analysis will be done to detect the gene mutation. To date, analysis of 100 families for DFNB6 locus has been completed and an Azeri family has been linked to *TMIE* gene. Further analysis and mutation detection is underway.

Key words: Non-syndromic hearing loss, DFNB, Iran.

P08.52
Fast approximate test for genome-wide association studies based on population data

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Genome-wide association (GWA) analysis is a powerful tool for identifying loci affecting complex traits. While standard association tests assume independence across samples, several approaches account for non-independence expected in presence of genetic substructure. The mixed models are among the most powerful methods allowing to account for relationship structure of the sample. However, application of mixed models to GWA scans is computationally demanding, and as a rule the computational time shows quadratic dependency on the number of subjects, requiring application of high performance computing when thousands of subjects and/or multiple traits are studied. Previously, two approximate methods, FASTA/mmScore (Chen and Abecasis, 2007; Aulchenko et al., 2010) and GRAMMAR (Aulchenko et al., 2007), have been proposed. mmScore gives unbiased estimates of model parameters, but computational time is quadratic on number of subjects, while GRAMMAR shows linear dependency, though results in biased effect estimates and conservative test statistics.

Here we propose new approximate method, which leads to unbiased effect estimates, has proper distribution of the test statistics, and is as fast as GRAMMAR. Our method is based on transformation of a vector of dependent phenotype residuals into a vector of independent ones by application of the matrix approximation allowing reduction of an effect of a covariance matrix to an effect of scalar factor. We derived an analytical formula for this scalar factor, making possible to apply simple linear regression method to estimate the effects and test statistics. This scalar factor is an analytical correction to GRAMMAR approach, which eliminates its conservatism and bias.

P08.53
Genome-wide association study to identify new genes/pathways conferring risk to OA susceptibility.

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Osteoarthritis (OA), also referred to as degenerative disease of the synovial joints, is caused by cartilage degradation going together with osteophyte formation and subchondral sclerosis causing pain and disability. To date, no cure is available and treatment is limited to pain relief.

OA is considered a complex disease in which both genetic and environmental factors contribute to its development. The estimated heritability differs per joint site and is estimated between 40 to 65%. It has been proposed, however, that this genetic component is highly polygenic with multiple risk alleles conferring small effects. Nevertheless, few consistent OA susceptibility genes emerged to date. Deep phenotyping may help to stratify OA into genetically and environmentally homogeneous subsets, thereby enhancing power and allowing identification of variants with larger impact on specific disease aspects.

Here, we set out to identify OA susceptibility loci conferring risk to generalized OA in the GARP study by genome wide association (GWAS). In the GARP study we have collected sibling pairs with symptomatic OA at multiple joint locations. We have identified 20 putative OA susceptibility loci with p-values $\leq 1*10^{-5}$ that are now being replicated both *in silico* (3 OA cohorts) and by *de novo* genotyping (1 OA cohort). In addition to the identification of loci conferring risk to OA, data obtained in the GWAS will be used for the identification

of pathways or groups of genes involved in the development of OA. Together, our results can contribute to a better understanding of OA pathology as well as its treatment.

P08.54
The Gln/Arg of human Paraoxonase polymorphisms (PON1 Leu55Met and Gln192Arg; PON2 Ser311Cys) is not related to acute myocardial infarction in the Tunisian population.

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Introduction: Paraoxonases (PONs) are closely related antioxidant enzymes encoded by clustered genes on chromosome 7q. Two particular polymorphisms, namely PON1-192 and PON2-311, in the genes encoding the antioxidant enzymes paraoxonase-1 (PON1) and paraoxonase-2 (PON2) have been associated with an increased risk of acute myocardial infarction (AMI). However, previous findings have been contradictory. We evaluated three PON polymorphisms (PON1 Leu55Met and Gln192Arg; PON2 Ser311Cys) in Tunisian patients with AMI.
Methods: 168 AMI patients compared to 169 healthy volunteers.
Results: PONs allele and genotype frequencies did not differ between patients and controls.
The PON polymorphisms (PON1 Leu55Met and Gln192Arg; PON2 Ser311Cys) were not significantly associated with AMI (p=1.11, p=0.09, p=1.46 respectively). No significant differences in age, sex, BMI, waist circumference, total Cholesterol, HDL-C and LDL-C were detected among the three-genotype subgroups of PON1 Leu55Met, PON1 Gln192Arg and PON2 Ser311Cys in the AMI patients.
Conclusions: The PON1 Leu55Met, PON1 Gln192Arg and PON2 Ser311Cys polymorphisms are not related to acute myocardial infarction in Tunisian population

P08.55
Genome-wide association study of the PolyCystic Ovary Syndrome: distribution of promising gene variants associated with testosterone in the LURIC cohort

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PolyCystic ovary syndrome (PCOS) causes hormonal imbalances affecting fertility, and metabolic diseases like obesity, type 2 diabetes, cardiovascular problems and quality of life. A genetic background of the PCOS is supported by affected families. Aim of the study is the detection of new genetic pathways for potential diagnostic and therapeutic targets in PCOS.
PCOS phenotype definition was based on hormonal and metabolic variables in women in comparison to men. Our Genome-Wide Association Study (GWAS) focuses on genetic alterations of a special PCOS phenotype, regarding high testosterone levels in female and male LUdwigshafen RIsk and Cardiovascular (LURIC) patients.
In total, 908.398 single nucleotide polymorphisms (SNPs) were generated as GWAS raw data. Those SNPs have been analyzed following multiple steps and have been annotated with different criteria for autosomal and X chromosome-linked genes, separately, using WGAViewer Software. The significant associations between SNPs at 17 loci in LURIC female and 9 loci in the whole cohort with high levels of testosterone were identified.
With the replication results of these candidate SNPs in our 669 carefully phenotyped PCOS and 410 control subjects, we expect to identify genetic variants contributing to diagnosis and new therapeutic aspects for PCOS prevention and treatment.

P08.56
Impact of polygenic profile to the performance of endurance and strength/power athletes

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We investigated five genetic polymorphisms that are candidates explaining individual variations in human physical performance phenotypic traits (*ACE* I/D (rs1799752); *ACTN3* C/T (rs1815739); *PPARGC1A* G/A (rs8192678); *PPARA* G/C (rs4253778); *PPARG* C/G (rs1801282)) in professional Lithuanian athletes (n=193) and in the general population of Lithuania (nonathletic controls, n=250). According to Williams and Folland (*J Physiol* 586:113-121, 2008) we calculated (1) the 'total genotype score' (TGS, the combination of five polymorphisms with the maximum value of '100' for the theoretically optimal polygenic score) in the athlete groups and in the Lithuanian population; and (2) the probability for the occurrence of Lithuanian individuals with the 'perfect' polygenic physical performance phenotype (power-oriented, endurance-oriented) profile. The TGS was calculated for the "power" and "endurance" groups of the Lithuanian athletes. We found the mean TGS significantly higher for the elite power-oriented athletes (44.4±11.3) compared to controls (33.6±13.2) ($p<0.05$) indicating more favorable polygenic profile for power-oriented athletes. No significant differences were found comparing the athletes in the endurance group (65.7±13.9) and controls (66.4±13.2). The obtained probability of a Lithuanian individual possessing a theoretically optimal endurance-oriented polygenic profile for up to five candidate genetic polymorphisms, equals to 1% (or 1 among 99 Lithuanian individuals); the optimal power-oriented polygenic profile accordingly 0.0007% (or 1 among 132650 Lithuanian individuals). The optimal combination of genotypes may occur more frequently in a very large population. Although, if there was a larger number of polymorphisms included in the analysis, the probability of possessing the optimal polygenic profile would diminish.

P08.57
Association of COMT and MTHFR polymorphisms with cognition in schizophrenia

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Objectives: The investigation of the effect of COMT (Val108/158Met) and MTHFR (C677T) polymorphisms on cognitive function in schizophrenia.
Material and Methods: 92 patients with chronic schizophrenia and 61 controls were assessed on cognitive functioning using the Wechsler Adult Intelligence Scale-(WAIS-III) and the Cambridge Neuropsychological Test Automated Battery (CANTAB). The CANTAB tests measured: speed of movement (MOT), pattern and spatial recognition memory (PRM, SRM respectively), spatial working memory (SWM), planning (SOC) and cognitive flexibility (IEDSS). RFLP analysis was performed to allow characterization of COMT and MTHFR polymorphisms.
Results: Participants were divided into four groups according to their genetic polymorphisms [VALC (Val-Val & CC), VALT (Val-Val & CT or TT), METC (Val-Met or Met-Met CC), METT (Val-Met or Met-Met & CT or TT)]. Positive correlations of COMT and MTHFR polymorphisms to cognition were recorded in both the group of patients with schizophrenia and the control group. Patients with VALT polymorphisms presented higher full scale and performance IQ than patients with VALC [$F(1, 42)=5.803$; $p=0.020$ and $F(1,42)=4.339$; $p=0.043$, respectively]. In all patients there was also a significant main effect of COMT in SOC, with those carrying VALC having a worse performance compared to the ones with METC [Kruskal-Wallis ($df=2$)$=6.828$, $p=0.033$]. Finally, in respect to PRM, SRM and SWM controls carrying VALT performed

better than those with VALC.
Conclusion: The above findings support the association of COMT and MTHFR genes with the cognitive performance in schizophrenia and are consistent with a possible epistatic nature of the polymorphisms studied.

P08.58
molecular analysis of Prader-Willi and Angelman syndromes: A study of twenty Tunisian patients

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Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are neurobehavioral disorders that result from the loss of expression of imprinted genes in the paternal and maternal chromosome 15, respectively.
The aim of this work is to achieve a molecular study for 20 patients (7 AS; 13 PWS) in order to determine the exact molecular mechanism involved.
A positive methylation-specific PCR (MS-PCR) enabled the reliable diagnosis of 13 PWS and 7 AS patients. For all these cases, we applied FISH technique. Thereafter, we have developed a linkage analysis method by testing 8 polymorphic genetic markers within the PWS/AS critical region 15q11q13.
FISH study allows us to classify our 20 subjects in two groups: 6 of 13 PWS (46%) patients had microdeletion of paternal 15q11-q13 and 6 of 7 AS patients (85%) had microdeletions of maternal 15q11-q13. The genotype and linkage analyses of the 8 non deletional probands and their family disclosed that Angelman patient had inherited two paternal alleles of chromosome 15 and 5 of 7 non deletional PWS (38%) subjects had a maternal uniparental disomy of chromosome 15 (UPD 15). Among these PWS patients, 4 cases had a maternal heterodisomy and one had a maternal isodiomy. Moreover, and despite the abnormal methylation patterns of the 15q11-q13 region, the 2 remaining PWS cases presented none of the alterations shown by the above groups.
A combination of these methods is important for the identification of genetic alterations involved in our PWS or AS patients and the assessment of the risk of recurrence.

P08.59
Investigation of candidate loci for primary open angle glaucoma in a Brazilian family through SNP array.

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Glaucoma is one of the major causes of irreversible blindness worldwide, characterized by progressive loss of optic nerve ganglion cells, associated with correspondent visual field damage. Among several forms of glaucoma, the most prevalent is the primary open angle glaucoma (POAG). The increase of intraocular pressure is the most important risk factor for the development of POAG. There are at least 14 loci (*GLC1A* - *GLC1N*) associated with POAG identified from genetic mapping studies, most of them involving families that follow a Mendelian inheritance pattern but only three genes were identified. The first described gene was myocilin (*MYOC* - *GLC1A*), followed by optineurin (*OPTN* or Optic Neuropathy Induced Protein - *GLC1E*) and WD Repeat-Containing Protein 36 (*WDR36* - *GLC1G)* genes. The purpose of this study was to evaluate regions in the genome associated with glaucoma through linkage study in one POAG informative family, through single nucleotide polymorphism (SNP) microarray. Comprehensive ophthalmic evaluation was performed and genomic DNA obtained from one Brazilian family (19 individuals) with POAG. Genome-wide linkage analysis was conducted using the Affymetrix 10K SNP array and analyzed by JINGLEFIX and MERLIN softwares. The SNP array generated 8.962 valid SNPs. Three chromosomal regions (1p, 5q and 14q) with lod scores equal to 1.9, 2.1 and 1.8, respectively were observed. Although the results do not show values of lod scores more than 3.00, these three regions are of great interest because they will be refined with microsatellites markers to identify potential susceptibility genes for POAG.
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P08.60
BAFF and FCGRIIIA polymorphisms are predictive factors of response to rituximab in Rheumatoid Arthritis (RA)

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The presence of RF or ACPA was previously reported as predictive factors of response to rituximab (RTX) in RA. SMART is a randomized open trial assessing 2 strategies of re-treatment in patients (pts) responding to RTX after failure or intolerance to anti-TNF therapy. The objective of this ancillary study was to identify genetic markers predictive of response to a first course of RTX. The potential association between single-nucleotide polymorphisms (SNP) in 10 candidate genes involved in B cell pathways and response to RTX was investigated. Baseline clinical and biologic factors were collected. Univariate and multivariate analyses were performed to identify predictive factors of good or moderate EULAR response in comparison with non response at week 24. Among the 115 pts (age = 56±11 years, 80% of women, 14±9 years of disease duration, baseline DAS28 = 5.7±0.9, 70%RF+, 82% ACPA+), 93 (81%) were responders. One non-synonymous coding SNP (rs396991) of FCGRIIIA (1q23) and one SNP (rs9514828) in the 5' regulatory region (-871 C/T) of BAFF (13q32-q34) were associated with response to RTX. For FCGRIIIA, G allele carriers had higher response rate (91% of responders versus 70%, OR=4.6 [95%CI: 1.5-13.6], p=0.006) in comparison to non carriers. For BAFF, C allele carriers had higher response rate (85% of responders versus 64%, OR=3.2 [1.1-9.1], p=0.03) in comparison to non carriers. These results were confirmed in multivariate analysis. In RA pts failing to anti-TNF, two polymorphisms of FCGRIIIA and BAFF appear as additional factors associated with higher response rate after a first course of RTX.

P08.61
PredictABEL: an R package for the assessment of risk prediction models

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The rapid identification of genetic markers for multifactorial diseases from genome-wide association studies is fuelling interest in investigating the predictive ability and health care utility of genetic risk models. Various measures are available for the assessment of risk prediction models, each addressing a different aspect of performance and utility. Even though the assessment of risk prediction models is relatively standard, there is no single statistical package that would allow for the computation and production of all these measures and plots. Therefore, we developed PredictABEL, an R package that contains functions to obtain all descriptive tables, measures and plots that are used in genetic risk prediction studies. The package contains functions for the various measures that are used in empirical studies, including univariate and multivariate odds ratios (OR) of the predictors, the c-statistic (or area under the receiver operating characteristic (ROC) curve (AUC)), Hosmer-Lemeshow goodness of fit test, reclassification table, net reclassification improvement (NRI) and integrated discrimination improvement (IDI). Also included are functions to create plots, such as risk distributions, ROC curves, calibration plot, discrimination box plot and predictiveness curves. In addition to functions to assess the performance of risk models, the package includes functions to obtain weighted and unweighted risk scores as well as predicted risks using logistic regression analysis. These logistic regression functions are specifically written for models that include genetic variables, but they can also be applied to models that are based on non-genetic risk factors only. PredictABEL is freely available at the CRAN website (http://cran.r-project.org/).

P08.62
Identification of novel mutations in CRB1 and CERKL genes by genomewide homozygosity mapping in Turkish families with autosomal recessive retinitis pigmentosa

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Retinitis pigmentosa (RP), the most common form of inherited retinal dystrophies, shows extremely high clinical and genetic heterogeneity. To date, more than 35 genes have been associated with this disease in all modes of inheritance. Although the number of RP causative genes is continuously expanding, more than 40% of the cases remain undefined. All available mutation detection techniques of all known RP genes remain labor intensive, time consuming, and expensive. To overcome these challenges and to generate a high volume, cost-effective, and efficient screening tool, microarray which is high-throughput molecular screening techniques has been prefered.

Whole genome genotyping was performed by Affymetrix 250K SNP array in two consanguineous Turkish families in which two children have RP. Homozygosity mapping have shown linkage to chromosome 1 and chromosome 2 in these families respectively. RP causing CRB1 and CERKL genes located in these chromosomal region was selected as a first candidate gene for mutation screening. The mutation screening in these genes revealed the presence of two novel mutations: 701dupACAGG(fs)□.710X in CRB1 gene, IVS1+5G>C in CERKL gene.

Mutation frequency survey of different populations for CERKL and CRB1 mutations showed that they account for 1% of all arRP associated gene mutations. Genomewide genotyping approach is a useful in selection of candidate genes among many disease causing RP genes. This study was supported by Scientific Research Fund of Hacettepe University (BAP Project No: 0801601003).

P08.63
Genome-wide association study of saliva flow rate and integration of protein-gene networks

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Background/Objective: Due to limited health care access and elevated oral disease, the Center for Oral Health Research in Appalachia (COHRA) began evaluating risk factors for dental caries in families who were also included as part of the NIH Gene, Environment Association Studies Consortium (GENEVA) genome-wide association study (GWAS) of dentalcaries. Dental caries is heritable (29%~40%) even after adjustments for shared environment. Saliva flow may impact caries risk and be under control by multiple genes. We integrate GWAS of saliva flow with existing protein databases to identify corresponding gene networks in the COHRA-GENEVA population.

Methods: Caries status, demographics, and unstimulated saliva flow rates (ml/min) were obtained from COHRA-GENEVA subjects (n = 1506; 647 males and 859 females). Subjects self-reported as Caucasian, and were 5-75 years old. Heritability of saliva flow was estimated at 49%. GWAS was conducted of unstimulated saliva flow (mean=0.581(ml/min), sd=0.418). Illumina 610-Quad platform genotypes were generated by the Center for Inherited Disease Research. Genotype quality control was conducted by the GENEVA Coordinating Center and U. Pittsburgh using standardized protocols. Single-SNP GWAS results were integrated into dmGWAS software to identify genetic modules based on joint consideration of SNP results and existing 63,995 gene pairs from the Protein Interaction Network Analysis (PINA) database.

Results: Single SNP GWAS results suggest regions of chromosomes 2, 8, 9, 10, 13 and 22 may be associated with a saliva flow rate. We are currently applying pathway-integration approaches to GWAS data to prioritize gene webs influencing saliva flow.

P08.64***
Next-generation sequencing of known and putative susceptibility genes for schizophrenia and autism spectrum disorders to detect rare high-penetrant risk variants

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Schizophrenia (SZ) and autism spectrum disorders (ASD) are complex neurodevelopmental disorders that share certain phenotypes including cognitive deficits and some behavioural characteristics. Such similarities suggest that these disorders may share an underlying pathology and thus may share some genetic risk variants. This study involves next-generation sequencing of the exonic regions of 215 potential susceptibility genes in an Irish sample of 150 cases of ASD, 300 cases of SZ and 300 controls, in order to identify single nucleotide polymorphisms, indels and structural variants contributing to one or both disorders. A multiplex target enrichment method is used whereby DNA samples are multiplexed together using DNA indexes/barcodes and enriched for the exonic regions of these genes using the Agilent SureSelect target enrichment method. This is followed by 80bp paired-end sequencing in a single lane of an Illumina GAII. Gene selection comprised of five categories: 1) Interactors of NRXN1, 2) Interactors of DISC1, 3) Genes within the Glutamate Receptor Complexes; NMDA, mGluR5 and AMPA, 4) Cell adhesion molecules and 5) Functional and Positional Candidates. Analysis of the pilot set of samples indicates that the approach undertaken is successful with an even spread of sequence information for 24 indexed samples per lane, >8X coverage for 84% of target regions and overall SNP concordance with previous GWAS data (Affymetrix 6.0) of 99.3%. In addition to description of this novel sequencing method, data will be presented on the rare variant analysis in the SZ and ASD samples.

P08.65
Frequency of serotonin transporter promoter gene polymorphism in opioid addiction

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Background: Association studies of polymorphism in the promoter region (5′-HTTP) of the gene encoding the serotonin transporter protein in addiction have shown conflicting results. The most frequently observed alleles of the 5′-HTTLPR polymorphism are the short and long variants, which the short variant is associated with lower transcriptional activity than long variant. Aim of the present study was to evaluate the possible association between 5-HTT genotype and the availability to experiment illegal drugs among adolescents.

Methods: Thirty one opium dependent males, aged 20-50 years, entered the study, after informed consent. They referred to the rehabilitation centre to give up addiction. Thirty one normal volunteer were chosen for control, which were matched in age, sex and socioeconomic situation with cases. A whole blood sample (5 ml) from each participant was collected and stored for subsequent detection of DNA using phenol-chloroform method. DNA isolated from whole blood was PCR-amplified. The PCR products were resolved in 2.5% agarose gel containing 50 mg/ml ethidium bromide in TAE buffer (40 mM Tris-acetate, 1mMEDTA pH 8.0).

Results: Short allele frequency in cases and controls were 0.532 and 0.387 respectively. Long allele frequency in cases and controls were 0.468 and 0.613 respectively. Despite of OR=1.8 this study indicate no significant association between frequency of s allele and addiction to opioid.

Conclusions: Although previous findings indicated association of short allele with addiction, this study did not confirm these results. Several possibilities exist for these discrepant results, including small sample size, potential population stratification, and categorical phenotypes.

P08.66
TDT analysis used for searching new molecular markers involved in SMA disease

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Clinical heterogeneity of spinal muscular atrophy (SMA) disease suggests the involvement of some unidentified genetic factors, in addition to those already known. Taking into account the role of vitamin D in neuronal and muscular cells physiopathology, we think that vitamin D receptor (*VDR*) gene might be a candidate gene for SMA heterogeneity. The aim of our study was to asses, through a transmission disequilibrium test (TDT), the relationship between four vitamin D receptor markers and this ruthless disease.

We included in our study 30 SMA subjects (clinically and molecular diagnosed) and their both parents. These nuclear families were genotyped for *VDR* gene *FokI*, *BsmI*, *ApaI* and *TaqI* polymorphisms, by PCR-RFLP technique. TDT analysis realized with FBAT software showed that the "*t*" allele of *TaqI* polymorphism is transmitted from parents to affected offspring more often than statistically expected (p=0.04). For the other alleles, no statistically significant results were noticed. Also, we didn't identify the preferential transmission of any estimated four-locus haplotype. This is the first report about using TDT analysis in investigation of SMA disease. Even we analyzed a small number of nuclear families, we think that our preliminary results sustain the hypothesis of *VDR* gene involvement in this pathology, but extended research is needed to be performed in order to assess our results.

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P08.67
SNP array analysis in 4 patients in an attempt to identify the mitochondrial pyruvate carrier

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Transport of pyruvate into mitochondria is fundamental to glucose oxidation and ATP production. However, the mammalian transporter has
not yet been identified.

We previously reported a patient with encephalopathy associated to persistant hyperlactatemia and normal lactate/pyruvate ratios, in whom pyruvate dehydrogenase deficiency was ruled out by enzymatic assays and mutation screening. A defect of pyruvate transport across the mitochondrial membrane was considered as an alternative hypothesis.

Parallel methods in disrupted and digitonin-permeabilized fibroblasts were designed to measure the oxidation of [14C] pyruvate : digitonin allows pyruvate to bypass the plasma membrane and directly reach the mitochondrial membrane. We established that [14C]-pyruvate oxidation was normal in disrupted fibroblasts and severely impaired in permeabilized fibroblasts whose mitochondrial membrane was intact. Two other patients were further identified. All 3 families are of Algerian descent and consanguineous.

In an attempt to identify the disease-causing gene, homozygosity mapping using the DNA of the first two families (3 affected members) was performed on a GeneChip 250K SNP array from Affymetrix. Several common regions covering more than 35 contiguous homozygous SNPs were uncovered but neither corresponded to genes of the mitochondrial transporter family. DNA from the proband from the recently identified third family is being similarly analysed, and should restrict the number of candidate regions, assuming genetic homogeneity. This will also allow us to test, through haplotype analysis, the hypothesis of a founder effect, and search for the mutated gene.

P08.68
TRIB1 genes polymorphism in type 2 diabetes mellitus and metabolic syndrome patients

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Type 2 diabetes mellitus (T2DM) and metabolic syndrome (MS) have polygenic background. Characterized by gene-gene and gene-environment interactions with onset in adulthood, usually at age 40 to 60 but occasionally in adolescence if a person is obese. The penetrance is variable, possibly 10 to 40%

The human tribbles-1 (*TRIB1*) gene is located at chromosome 8q24. Hepatic-specific overexpression of TRIB1 reduces levels of plasma TG and cholesterol by limiting VLDL production. The rs17321515 was shown to be associated with severe HTG and HLP types 2B, 3, 4 and 5. In Asian Malay population, this polymorphism located on the *TRIB1* locus showed an association with elevated total- and LDL-cholesterol and with increased risk of CHD and CVD and also, in a Japanese cohort this variant was significantly associated with triglyceride levels and LDL-cholesterol concentrations. A total of 426 T2DM, 489 MS patients and 253 controls were genotyped by PCR-RFLP. We observed relationships between triglycerides and any genotypes in T2DM and MS patients. Triglyceride levels where higher in minor homozygotes than in two other genotype groups (T2DM: AA:2.12±0.28; AG:1.79±0.12; GG:1.99±0.21; MS: AA:2.49±0.18; AG:2.10±0.09; GG:2.15±0.16 and $p<0.05$). The total serum cholesterol and HDL-cholesterol level did not differ between groups of different genotypes. Multiple logistic regression analysis revealed that this genotype protect for the development of T2DM (OR=0.495; 95% CI:0.292-0.827; p=0.007). The analyzed TRIB1 (rs17321515) SNP is associated with elevated triglyceride levels in AA homozygotes in T2DM and metabolic syndrome groups and protect for development of T2DM.

P08.69
Associations of STAT3, JAK2 and CCR6 polymorphisms with ulcerative colitis and Crohn's disease.

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Recent genome-wide association studies have identified many genetic factors in association with the development of inflammatory bowel diseases ulcderative colitis (UC) and Crohn's disease (CD).In this study we investigated the possible association of the natural variants of the following genes: signal transducer and activator of transcription 3 (STAT3-rs744166), janus kinase 2 (JAK2-rs10758669) and chemokine (C-C motif) receptor 6 (CCR-rs2301436) in patients with UC or CD. DNA of 309 CD, 307 UC, and 496 control patients were genotyped by PCR/RFLP method. Although no significant differences were detected between the patient groups and the controls regarding the STAT3 T risk allele frequencies, we found a significantly higher frequency of rs744166 TT homozygotes in UC patients than in controls, while no such association could be observed in patients with CD. For the JAK2 polymorphism rs10758669, we found an elevated overall frequency of the C risk allele in CD patients but not in UC patients, while the frequecies of CC homozygotes did not differ significantly in the UC and CD groups compared to controls. As for the CCR6 rs2301436 variant, we detected a statistically elevated ratio of risk AA homozygotes in UC patients, but not in the CD group when compared to controls. Regression analysis revealed a risk nature of STAT3 rs744166 TT genotype for ulcerative colitis (OR 1.498, 95% CI 1.121-2.000; p=0.006, adjusted 0.009). On the other hand, in our cohort we could not detect such associations of JAK2 variant rs10758669 or CCR6 variant rs2301436 with UC or CD.

P08.70
Evidence of DLL1 as a susceptibility locus in Indian Visceral leishmaniasis

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Previous genome wide linkage studies in Sudanese and Brazilian populations identified chromosome 6q27 as a strong candidate region containing a gene(s) regulating susceptibility to visceral leishmaniasis (VL). The region contains genes involved in the host immune response, amongst which the most compelling candidate susceptibility gene for VL is *DLL1* (Delta Like Protein 1), a member of the notch signalling pathway that directs naïve T helper differentiation towards Th1, Th2 or regulatory T cell lineages.

This population-based study investigated the role of the 6q27 genes (*PHF10*, *C6orf70, DLL1*, *FAM120B*, *PSMB1*, *TBP*) in Indian VL by genotyping (Sequenom) twenty-one single nucleotide polymorphisms in 941 VL cases and 992 ethnically-matched controls. Logistic regression analyses using additive and genotypic models show significant associations with variants at *DLL1* (rs1884190) and *FAM120B* (rs9366198, rs9460106, rs2103816). FAM120B_rs9460106 (intronic) was the most associated SNP (OR=1.22; 95%CI=1.07-1.39; *P*=0.0027), but the associated variants all fall within a strong linkage disequilibrium block. Quantitative gene expression was analysed in 19 paired pre- (Day 0), and post- (Day 30) treatment splenic aspirate samples from VL patients receiving antileishmanial drug therapy. *DLL1* was significantly (*P*<0.0001) higher expression in Day 0 samples compared to Day 30.

These genetic and functional studies in an endemic Indian population provide strong evidence for *DLL1* acting as a susceptibility locus in India.

P08.71

Tru9I polymorphism in vitamin D receptor gene and bone mineral density association in different menopausal situation: Tehran lipid and glucose study

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Introduction: The vitamin D endocrine systems play a central role in control of bone and calcium homeostasis. Thus, alterations in the vitamin D pathway lead to disturbances in mineral metabolism. Several genetic variations have been identified in the VDR. The aim of this study was is to test whether there is an association between G/A polymorphism (Tru9I) in intron 8 and bone mineral density (BMD) and menopausal statues.

Material and methods: In this study 137 healthy free-living post menopausal women and underwent BMD randomly selected. Serum 25(OH) D, calcium, phosphorus and bone mass were measured (81 premenopausal; 56 postmenopausal). Hardy-Weinberg equilibrium analysis was used. To examine the selected polymorphism RFLP-PCR were used with Tru9I.

Result: The result showed that T allele frequency was 0.82. After adjustments for age, in premenopausal subjects mean BMD for femoral neck T carriers were higher than t carriers ($p < 0.05$) but the opposite results in postmenopausal women have been seen.

Conclusion: Our findings suggest that the VDR Tru9I polymorphism may be associated with BMD and age as a covariable play a significant role. These results highlight the importance of the genetic factors in the bone mineral density of woman in different hormonal stage.

P08.72

LD-based SNP genotype calling from next-generation sequencing data

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The 1000 Genomes project (1KGP) aims to construct a detailed catalogue of human polymorphism using next-generation sequencing technologies which consist of short reads of DNA mapped to a reference genome. These reads can be used for the detection of structural variation, indels and SNPs. However, it is costly to sequence many individuals at high depth. An alternative approach is to sequence many samples at low coverage and then combine the information across samples to detect and genotype variant sites.

Our method assumes that a scaffold of haplotypes at known SNPs is available, e.g. , from the HapMap project. At each potentially variant site we use a Gibbs sampling phasing scheme to update each individuals haplotypes at that site and known SNPs in small flanking window. Rather than using an HMM in the phasing updates we use a simple, fast and novel method based on approximating a new haplotype as a draw from an appropriately chosen multivariate normal distribution. Since the haplotypes at the known sites are static many of the calculations involved can be efficiently implemented so that the method is fast and scales well. The evidence for variation at the site and estimates of individual genotypes can be obtained by summarizing the MCMC samples.

We have applied our method to a set of CEU individuals from Pilot 1 of the 1KGP and we compare our SNP and genotype calls with other existing methods. The number of SNP calls made and the genotype accuracy is comparable with other methods.

P08.73

Screening of 10 SNPs of LINGO1 gene in patients with essential tremor in Latvian population

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Essential tremor (ET) is one of the most common neurological disease, with a prevalence (age ≥ 40 years) estimated to be 4.0 % and prevalence in the oldest exceeding 20.0 %. An autosomal dominantly inherited form of ET is genetically linked to two loci on chromosomes 3q13 (ETM1) and 2p24.1 (ETM2) in families from different parts of the world. Numerous of candidate genes, including hypothetical, - HS1-BP3, HCLS1, DRD3, LINGO1, LINGO2, C2orf43, LOC 100129278, FLJ 21820, LOC 645949 - have been suggested during last years, but none of them was maintained up to now.

A marker within the LINGO1 gene, rs9652490, showing significant genome-wide association with ET, was recently reported in an Icelandic and North American populations. To replicate this association in an independent population from Latvia, we sequenced regions of LINGO1 gene involving 10 markers (rs2137110, rs8030859, rs34476171, rs9652490, rs7177008, rs13313467, rs8028808, rs11856808, rs72744599 and one novel SNP) in 141 unrelated Latvian ET patients (sporadic n = 64 and familial n = 77) and 130 normal controls, matched by age and gender.

We observed significant association with A/G genotype of the marker rs96532490 in familial ET compared to controls (P = 0.0426, OR = 1.92, 95 % CI: 1.06 - 3.46). No other significant association was found. Our results suggest that the LINGO1 variants analyzed are not a major risk factor for developing ET in Latvian population, which propose the existence of other unknown genetic risk factors responsible for ET phenotype.

P08.74

Selection of endophenotypes in association mapping

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Most common diseases such as hypertension, diabetes, asthma, schizophrenia, heart diseases, etc. have a complex etiology involving both genetic and environmental factors. This complexity challenges investigators in finding the relationships between phenotypes and genotypes. One option to dissolve the complexity is to select an “endophenotype” to signify quantitative measures that are more proximate to the biological etiology of a clinical disorder than its signs and symptoms and thus less genetically complex than the disorder’s underlying mechanism. Since the endophenotype is influenced by fewer genetic and environmental risk factors than the disorder, its use would result in greater statistical power to detect the effects of individual genes. In practice, it is common for investigators to measure numerous phenotypes on affected individuals, to achieve a substantial possibility of success in gene mapping, a systematic statistical method on the selection of endophenotypes would be helpful on identification of disease genes. In this study, we applied a developed statistical approach to select valid endophenotypes in linkage or association mapping. This approach allows investigators to assess the significance of an endophenotype on a pathway as incorporating a proper endophenotype into gene mapping can enhance the efficiency of disease locus estimate. This approach is applicable to all kinds of study designs in association mapping and linkage mapping.

J08.01

Screening for *MYOXVA* gene mutations of DFNB3 locus in Autosomal recessive non-syndromic, *GJB2* negative Iranian Deaf population

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Myo15A gene is located in DFNB3 locus on chromosome 17p11.2, and encodes Myosin XVA, which is an unconventional myosin and is critical for the formation of stereocilia in hair cells of the inner ear. Recessive mutations in this gene can lead to profound Autosomal recessive non-syndromic hearing loss (ARNSHL) in humans and *shaker2* (*sh2*) phenotype in mice.

Here, we performed a family study on 140 Iranian families in order to determine mutations causing ARNSHL. The families, who were negative for *GJB2* gene mutations, were subjected to linkage analysis. Eight of them were linked to DFNB3 locus using intragenic or flanking STR markers. This data suggests that the frequency of *Myo15A* mutation is about 5% in the Iranian deaf population. Sequencing of *Myo15A* in these families led to the identification of five unreported mutations - 3 missense mutations, 1 nonsense mutation and 1 deletion which results in frame shift mutation followed by premature stop codon.

J08.02

G-2548A polymorphism of the human leptin gene (LEP) promoter region associated with the level of serum lipoprotein

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Leptin - a peptide hormone that is secreted by fat cells and is involved in the regulation of energy metabolism and body weight. It reduces appetite, increases energy expenditure, changes the metabolism of fats and glucose, as well as neuroendocrine function or direct influence, or activation of specific structures in the central nervous system.

Gene LEP, encodes the protein leptin is localized on 7q31.3.

We have studied the polymorphic variant G-2548A promoter region of the gene LEP.

Material for the study included DNA samples from 350 individuals. Determining the level of low density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol by standard enzymatic methods. Analysis of polymorphic loci LEP G-2548A was performed by PCR-RFLP.

Results: The group of persons with indicators of LDL in the normal range was a statistically significant increase of homozygous genotype LEP *G/*G (27.34% against 15.38% in the group of persons with high levels of LDL, P= 0.0192).

Also in the group of persons with higher than normal rates of HDL showed a significant increase of genotype LEP *G/*G (37.5% vs. 24.03%, P=0.0407) and allele LEP *G (60.94% against, 49.65%) against a group of individuals with indicators of HDL cholesterol within normal limits.

Conclusions: The findings suggest that the allelic variant G-2548A contributes to the determination of lipid metabolism. In particular, the residents of the Republic of Bashkortostan, a favorable genetic marker serum levels of LDL and HDL may be LEP *G allele and genotype of LEP *G/*G.

J08.03

Association of the G72/G30 genes with psychiatric disorders

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Earlier studies have shown the significant role of G72 and G30 genes in the pathogenesis of many psychiatric disorders. The present study is aimed at the analysis of the association of the rs3918342, rs2391191 and rs978714 of G72 gene and rs1341402 of G30 gene with schizophrenia and affective disorders. DNA samples of 144 schizophrenia patients and 129 affective mood disorders patients from Bashkortostan (Russia) have been used as the material for this study. The control group comprised 165 volunteers of the same age and ethnic background not registered in any mental health or drug abuse clinics and denying psychiatrically burdened family history. Case-control comparisons were based on association analysis, linkage disequilibrium and haplotype frequency estimations. The C allele (rs3918342) associated with an increased risk of schizophrenia (OR=1,49) and affective disorders (OR=1,46). Maximum likelihood analysis of haplotype distribution demonstrated the presence of linkage disequilibrium between the two loci rs2391191 and rs1341402. The case group with schizophrenia showed bigger CG haplotype frequency compared to that in the controls, however, the difference has not reached the statistical significance (P=0,081).

J08.04

VEGF gene polymorphism association with diabetic foot ulcer

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Objective: Functional polymorphisms within vascular endothelial growth factor (VEGF) gene have shown association with various conditions including diabetic neuropathy and retinopathy. In this study we have performed a candidate gene association study in order to examine VEGF gene polymorphism association with diabetic foot ulcer (DFU).

Methods: The study group comprised of type 2 diabetes patients with (N=247) and without (N=241) DFU. Healthy control subjects (N=98) were also recruited from the same area. The ARMS-PCR technique was applied for genotyping of VEGF gene SNPs at positions -7*C/T and -2578*C/A.

Results: The frequency of genotype AA was significantly decreased in patients with DFU compared with diabetic subjects without DFU (AA vs CA+CC, p=0.003, OR= 0.44, CI = 0.24-0.80.). Also there was a significant decrease in frequency of A allele in patients with DFU compared to the controls (p=0.02, OR= 0.68, CI = 0.48-0.96).

Conclusion: It seems that lower frequency of A allele in patients with DFU is conferring a protective effect which might be as a result of increased angiogenesis in patients carrying this allele.

Table . VEGF polymorphisms frequencies in Controls (C), T2DM (P) and diabetic foot ulcer (DFU)

VEGF -2578*C/A	**C n (%)**	**P n (%)**	**DFU n (%)**	**P Value**		
				DFU/C	**DFU/P**	**P/C**
Genotype CC	22 (22.4)	63 (29.0)	68 (27.5)	**0.003**	0.1	0.1
CA	47 (48.0)	108 (49.8)	140 (56.6)			
AA	29 (29.61)	46 (21.2)	39 (15.7)			
Allele						
C	91 (46.0)	234 (53.9)	276 (56.0)	**0.02**	0.5	0.08
A	105 (54.0)	200 (46.0)	218 (44.0)			
VEGF -7*C/T						
Genotype CC	72 (75.8)	158 (71.8)	186 (77.8)	0.1	0.07	0.9
CT	22 (23.2)	38 (17.3)	43 (17.9)			
TT	1 (1.1)	2 (0.9)	10 (4.0)	0.8	0.2	0.4
Allele						
C	166 (87.3)	354 (89.3)	415 (86.8)	0.8	0.2	0.4
T	24 (12.6)	42 (11.7)	63 (13.1)			

J08.05
AGTR1 c.*86A>C and AGT c.620C>T polymorphism genotypes and the aerobic capacity of elite Lithuanian athletes

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Angiotensinogen (AGT) and angiotensin II type 1 receptor genes (AGTR1) are important for the angiotensin- renin system which is crucial for the function of the cardiovascular system and affects ones physical capacity. We analyzed AGTR1 c.*86A>C polymorphism which is located in the 3â€~UTR and has major impact on gene transcription and mRNA stability. The angiotensinogen gene (AGT) regulates the expression of AGT (angiotensin II precursor) in the liver. The missense AGT c.620C>T polymorphism might have serious impact on the production of angiotensin and subsequently an athletes physical capacity.

The statistical analysis of the polymorphisms and their association with physical capacityâ€™s phenotypical index VO2max in a group of 149 elite Lithuanian athletes showed their significant association. The athletes were stratified into three groups (speed/power, endurance and mixed sports groups) according to the sport propagated. 240 unrelated subjects from the general Lithuanian population were used as a control group.

Statistical analysis revealed no significant VO2max differences between athletes with different AGT c.620C>T polymorphism genotypes. AGT C/C genotype was significantly more frequent in the endurance group compared to the speed/power group. The AGTR1 A/A genotype athletes have statistically significantly higher VO2max index than the C/C genotype athletes. C allele of the AGTR1 A/C polymorphism was significantly more frequent in the speed/power group compared to other sports groups and the control group. We conclude that while both AGTR1 and AGT polymorphism genotypes indicate tendancy towards a certain sport type, but only AGTR1 A/C polimorphism genotypes could be used for VO2max index value size prediction.

J08.06
Existence of association between P86L polymorphism in *CALHM1* gene and risk of developing late-onset Alzheimer's disease in the Iranian population

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Introduction: Alzheimer's disease (AD) is a genetically heterogeneous neurodegenerative disease and Late-Onset type (LOAD) is the most common form of dementia affecting people over 65 years old. AD is a complex disease with multifactorial etiology. This disease is characterized by hippocampal atrophy and cerebral Aβ peptide deposition. *CALHM1* gene is located on 10q24.33 and encodes a multipass transmembrane glycoprotein that controls cytosolic $Ca2^+$ concentrations and Aβ levels. *CALHM1* P86L polymorphism (rs2986017) is significantly associated with LOAD in independent case controls in a number of studies. This polymorphism increases Aβ levels by interfering with CALHM1 mediated $Ca2^+$ permeability.

Aims: This study was performed to determine whether this polymorphism contributes to the risk for LOAD in Iranian population.

Methods and Materials: One hundred and forty one AD patients and 141 healthy controls were recruited in this study. After extraction of genomic DNA, the genotype and allele frequencies were determined in case and control subjects using PCR/RFLP method.

Results: The statistical analysis showed a significant difference in the heterozygote genotype frequency in case and control groups and polymorphic allele had a protective role between two groups. Also after stratifying the subjects by their *APOE-ε4* status, no significant association was observed.

Discussion: Our study suggests that P86L polymorphism could be a risk and protective factor for late-onset Alzheimer's disease (LOAD) in Iranian population. However, to confirm these results, further study with a bigger sample size may be required.

Keywords: Alzheimer's disease, Polymorphism, Association study, Population, *CALHM1* gene.

J08.07
Association of LOC387715 gene polymorphism with both forms of advanced age-related macular degeneration in Turkish patients

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Age-related macular degeneration (AMD) is a clinically heterogeneous disorder and the leading cause of irreversible central vision loss in the elderly population. Although AMD etiology remains elusive, many environmental and genetic risk factors have been shown for AMD. A69S polymorphism (rs10490924:G>T) in *LOC387715* gene on chromosome 10q26, is known to increase the risk of developing AMD. The purpose of this study is to investigate whether the one of the major risk allel in the *LOC387715*, were associated with advanced AMD in Turkish patients.

A total of 182 individuals, including 95 individuals with unrelated late AMD patients and 87 age-matched healthy individuals as a control group were genotyped by direct sequence analysis. Single SNP for A69S genotyping results were analyzed for deviations from H-W equilibrium and no significant deviations were detected ($p<0.05$; controls, p=0.92 ; cases, p=0.23). Odds ratios and 95% confidence intervals were calculated by logistic regression method. Findings showed that homozygote individuals with TT genotype have an increased risk (OR=4, 1.52-10.52%) for AMD when compared with GG genotype in Turkish population. This study suggest that the LOC*387715* polymorphism has significantly associated for both types of end-stage AMD in Turkish populations.

J08.08
*Ban*I/D13S141/D13S175 represents a novel informative haplotype at the *GJB2* gene region in Iranian population

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Non-syndromic sensorineural hearing loss (NSHL) represents the most cause of hearing loss in Iranian patients. In view of the large numbers of mutations identified in *GJB2*, mutations analysis of the gene has been time-consuming and cost-effective. Alternatively, molecular markers which are highly linked to the *GJB2* gene have proven to be useful in carrier detection and prenatal diagnosis of NSHL families. These markers usually show a population dependent based haplotype frequency. However, to date, no information on the genotyping and frequency of the markers is present for the Iranian population. In the present study, genotyping and the haplotype frequency of three markers including *Ban*I, D13S141 and D13S175, at the *GJB2* region were investigated. The haplotype frequency was estimated using PHASE program. The input data contained two alleles (+ and -) for *Ban*I, four alleles for D13S141 and seven alleles for D13S175. Among the 42 possible haplotypes estimated, four haplotypes showed relatively high frequency (≥5%). Therefore, a combination of *Ban*I/D13S141/D13S175 could be suggested as an informative haplotype for possible carrier detection and prenatal diagnosis of NSHL in Iranian population.

P09 Complex traits and polygenic disorders

P09.001
ACTN3 R577X polymorphism in Russian power athletes

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The alpha-actinin-3 (ACTN3) gene encodes a Z-disc structural protein which is found only in fast glycolytic muscle fibers. A common nonsense polymorphism in codon 577 of the ACTN3 gene (R577X) results in alpha-actinin-3 deficiency in XX homozygotes. Previous reports have shown a lower proportion of the ACTN3 XX genotype

in power-oriented athletes compared to the general population. In the present study we tested whether XX genotype was under-represented in Russian power-oriented athletes. The study involved 142 Russian weightlifters of regional or national competitive standard. ACTN3 genotype and allele frequencies were compared to 974 controls. The frequency of the ACTN3 X allele (30.3% vs. 38.3%; P = 0.011) was significantly different between weightlifters and controls. Although the frequency of the ACTN3 XX genotype (8.5% vs. 13.9%; P = 0.11) was not significantly lower in the whole group of weightlifters in comparison with controls, it was significantly lower (0%; P = 0.039) in highly elite athletes (n = 27), supporting the hypothesis that the presence of α-actinin-3 has a beneficial effect on the function of skeletal muscle in generating forceful contractions at high velocity. In conclusion, ACTN3 R577X polymorphism was associated with elite power athlete status in Russians.

P09.002
TOMM40 polymorphisms are associated with cardiovascular phenotypes

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TOMM40 gene which encodes the channel-forming subunit of a translocase of the mitochondrial outer membrane had been recently proved to be involved in Alzheimer's disease. In several GWA studies of lipid profile multiple signals had been detected with TOMM40 SNPs, although their independent role in cardiovascular pathology is still under discussion, mainly because of the strong linkage disequilibrium between TOMM40 and APOE.

In our study we genotyped several TOMM40 variants in a cohort with acute coronary syndrome (ACS, n=176) and healthy subjects (n=184). Association with a number of cardiovascular phenotypes was analyzed. In healthy women rs157580 was associated with the level of total cholesterol, very low-density lipoproteins cholesterol, low-density lipoprotein cholesterol, and triglycerides. In ACS group rs2075650 was associated with blood pressure parameters and low-density lipoproteins cholesterol. TOMM40 rs741780 was associated with coronary artery stenosis more than 50% (p=0.048), and rs1160985 with extracoronary stenosis (p=0.038). The progression of heart failure in 1 year follow up was associated with rs157580 (p=0.006).

These results demonstrate that TOMM40 may be involved in cardiovascular diseases.

P09.003
Genome-wide association study of acute stroke in Russia

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A number of previous genome wide association studies (GWAS) recently identified some genome variants to be associated with stroke in local populations. The aim of our study was to assess the individual risk of stroke development in Slavonic population.

We performed a case-control association study in 694 stroke patients from Moscow Slavonic population and 715 controls from the same population. Standard examinations including CT, MRI, duplex, Echo-CG, CTA, MRA were performed to identify the stroke cases. The stroke subtypes were diagnosed according to the TOAST criteria. Whole genome associative study was performed using DNA microarrays HumanCyto12 v. 2 ("Illumina", USA), allowing typed more than 300, 000 SNP and CNV regions also.

The resulting raw data were processed using software packages Genome Studio, PLINK, Helix Tree and eventually found several SNPs associated with the development of acute stroke. The most statistically significant association with the rs4578424 DNA marker, located on gene TUB locus on chromosome 15. This gene encodes a protein that participates in the system of signal transduction through G proteins, and may be associated with violation of lipid metabolism.

Our data suggest the association of rs4578424 on chromosome 15 with total stroke risk formation.

P09.004
Protective effect of the polymorphic variant at the IL2 gene in Polish patients with Addison's disease

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Autoimmune Addison's disease (AAD) is a rare enocrinopathy with several susceptibility loci. Polymorphic variants of the *IL2* and *IL2RA* genes have recently been described to confer risk for several autoimmune disorders. *IL2* encodes interleukin-2, involved in maintaining peripheral T-cell immune tolerance, whereas *IL2RA* encodes specific alpha subunit of its receptor. This study was aimed to investigate the associations of selected *IL2* (rs6534349 A/G, rs2069762 T/G, rs3136534 A/C) and *IL2RA* (rs11594656 T/A, rs2104286 A/G, rs7093069 C/T) single nucleotide polymorphisms (SNPs) with AAD in a Polish cohort. The analysis comprised 124 AAD patients and 436 healthy controls. Genotyping was performed by PCR-RFLP or PCR-SSCP.

The *IL2* rs3136534 C allele appeared significantly decreased in AAD compared to the control group (29.4% *vs.* 39.1%, p=0.005), yielding an OR of 0.65 (95%CI 0.48-0.88). The difference in distribution of its genotypes also revealed statistical significance (p=0.009). By contrast, the allele and genotype frequencies of five other SNPs did not present significant differences between patients and controls (p>0.05). Linkage disequilibrium measures revealed moderate linkage between investigated SNPs (D' 0.66-0.94, r^2≤0.25 for IL2, and D' 0.13-0.84, r^2≤0.33 for IL2RA). Three locus A-T-C *IL2* haplotype, including minor rs3136534 allele, was found significantly less frequently among affected subjects *vs.* controls (26.6% vs. 38.3%, p=0.0007). The analysis of inferred *IL2RA* haplotypes did not add any further information.

In conclusion, this study indicates plausible protective effect of the minor allele at rs3136534 in AAD. No association of AAD with other investigated *IL2* and *IL2RA* SNPs was found. Supported by grant N402359738.

P09.005
The genome-wide analysis of the ageing: the women only study.

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Background: As ageing is complex phenotype influenced by a variety of genes and molecular pathways, environment and stochastic factors, different approaches are needed to determine the underlying cause of these phenomena. The Estonian Biobank provides a very good opportunity to study frequency changes of the SNPs in different age groups. Whole genome SNP analysis using Illumina arrays (for example HumanCNV370-Duo and HumanOmniExpress) and a data-driven model in identifying genetic variants is more promising than scrutinizing the candidate genes. Concentrating only on females minimizes confounding effects resulting from differences between sexes.

Methods: The aim of the study was to examine allele frequency dependent discrepancies by conducting a genome-wide association study with >1.8 million HapMap2 Imputed and Quality Controlled SNP markers on 1500 Estonian women from different age groups (<40, 40-60, 60-80 and >83). To test the age dependence, the allele frequencies in all 4 age groups were compared using the Cochran-Armitage test for trend. Genome-wide significance was concluded for p-values less than 10^{-8}.

Results and Conclusions: Results suggest association between allele frequency differences in several loci, including 2p21, 2p16.3, 5q12 and 7q36.2 of different age groups as studied. These findings indicate to the loci, which include the alleles, which are increased and/or decreased in the oldest.

P09.006
Genetic dissection of Age-Related Macular Degeneration: evaluation of the impact of novel and known variations in the Italian polulation

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Age-related macular degeneration is a common ocular disease affecting more than one million of Italian people. Here we show the results of a large, multicentric case-control study aimed to discover new genetic variants associated with the disease and to confirm the effect of the known susceptibility polymorphisms in the Italian population. A number of 1157 Italian subjects were recruited and classified in three different categories: the first one includes patients with diagnosis of AMD (n=367), the second is composed by subjects clinically evaluated, without any sign of disease (n=330) and the last one includes subjects not clinically evaluated (general population, n=920). All subjects were genotyped for nine variants: del443ins54 and rs10490924 in *ARMS2* gene, rs1061170 and rs1410996 in *CFH* gene, rs9332739, rs547154, rs4151667 and rs2230199 in genes coding for complement proteins and rs2227306 in *IL8* gene. Our data disclose a new risk variant (rs2227306) located within *IL8* gene (p=0.007), confirm the major role of *ARMS2* (del443ins54, p=4.99*10^{-16}) and *CFH* (rs1061170, p=1.60*10^{-23}) on the susceptibility to AMD and rule out a major involvement of the other variations.

P09.007
Mutational analysis in paediatric patients with X-linked and autosomal recessive forms of ocular albinism

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Ocular albinism (OA) is a group of inherited disorders with reduced pigmentation of the eyes and associated decreased visual acuity, nystagmus, strabismus and photophobia. X-linked OA is caused by mutations of the *GPR143* gene, encoding a pigment cell specific receptor. Autosomal recessive ocular albinisms (AROA) represent clinically mild variants of the occulocutaneous forms of albinisms (OCA), where apart from ocular characteristics, hypopigmentation of the skin and hair is usually present. AROA may result from mutations in any of the genes responsible for OCA, namely *TYR*, *OCA2* and rarely *TYRP1* or *MATP*. Ocular albinism is difficult to differentiate clinically from other forms of albinism in young patients, especially in those with light complexion. Therefore, genetic analysis presents a possibility for an early definitive diagnosis.

Direct sequencing of the coding regions of the *GPR143, TYR* and *OCA2* genes were performed in 17 patients with clinical signs of ocular albinism aged 2 to 17 years. Four patients had X-linked OA due to mutations in *GPR143* gene (29%). Two detected mutations were novel (p.Gly296Arg, c.360+5G>A). Five patients had AROA due to mutations on both alleles of the *TYR* gene (29%). One patient had *TYR* mutation on only one allele and therefore this is not completely explaining the clinical characteristics. Three detected *TYR* mutations were novel (p.Tyr451Cys, p.Ala355Val, p.Asp197Asn). Surprisingly, none of the patient had AROA due to mutations in *OCA2* gene. Two patients had *OCA2* mutations in only one allele. In five patients no pathological mutations were detected (29%).

P09.008
GST M1 and GST T1 polymorphisms and their possible relation to alcohol dependence

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Alcoholism is a heterogeneous disorder with a multiple genetic background. Glutathione-S-transferases (GST) are polymorphic enzymes that belong to phase II metabolism and are responsible for detoxification of a large number of electrophiles compounds by conjugation with glutathione. The aim of this study is to investigate the clinical significance of GSTM1/T1 polymorphisms in alcohol dependence and their importance in relation to Lesch's typology. Genotype distributions of GSTM1/T1 deletion polymorphisms were examined in a sample of 96 patients with known history of alcohol dependence and 324 healthy individuals. GSTM1 non null genotype frequency was significantly higher in patient group compared to controls (64.6% *vs* 47.2%) and may be considered a risk factor related to alcohol consumption (OR=2.038; 95%CI [1.272-3.267], p=0.004). No significantly results were obtained in relation to the GSTT1 polymorphism. The conjugation of GSTM1/T1 non-null genotypes in the same individual does not increase the risk for alcoholism. Although, the GSTM1 non null genotype appears to be a susceptibility factor to alcoholism, only patients classified as Type III and IV according to Lesch's Typology show higher risk for alcohol dependence. This study is the first report about the GSTM1 polymorphism as a risk factor for alcoholism. Our results pointed to significantly differences in ethanol-preferring phenotype, as a human psychopathological state, and GST M1 polymorphism as a susceptibility gene for alcohol consumption, which probably indicated that this enzyme may increase tolerance to some alcohol metabolites involved in different biochemical pathways potentially linked to the risk for some additive behaviours.

P09.009
A sequence variation in the promoter of the placental alkaline phosphatase gene (ALPP) is associated with allele-specific expression in human term placenta.

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Placental alkaline phosphatase (PLAP), encoded by the ALPP gene, is produced by the fetal side of the placenta. This enzyme displays strong genetic variability with three common alleles giving rise to six common electrophoretic phenotypes, and a number of rare variants. Some of these variants were reported to be associated with pathology of pregnancy. We show here that the two most common ALPP allelic variants, *PI*^{1} and *PI*^{2}, differ in mRNA expression level. The differential expression was independent of the parental origin and probably results from linkage disequilibrium with sequence variation rs2014683G>A in the ALPP gene promoter that was shown to have allele-specific binding patterns to placental nuclear proteins. Allele-specific expression in human term placenta may account for association of some variants with pathology.

P09.010
Polyalanine repeat expansions in NIPA1 are associated with amyotrophic lateral sclerosis

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, which cause is still unknown. Genetic risk factors are thought to play a major role in its pathogenesis. Recent research has described deletions in NIPA1 increasing susceptibility for

ALS. Mutations in this gene are known to cause hereditary spastic paraparesis. In this study we investage whether missense mutations in NIPA1 and expansions its polyalanine repeat might play a role in ALS as well.

Methods: DNA samples were collected from 2293 ALS patients and 2777 healthy controls, of three different populations (Dutch, Belgian, German). All exons of NIPA1 were sequenced and fragment analysis was performed to determine the polyalanine repeat length. Alleles were grouped according to their length, with short alleles consisting of 13 alanines respectively.

Results: Sequencing reveiled 7 missense mutations in patients and 6 in controls (p = 0.59). In all populations the long repeat occured more frequently in patients, 2.82% compared to 1.80% in controls (OR = 1.63, p = 0.0007). Patients with these long alleles had a worse median survival (HR 1.58, p = 0.0001) and an earlier disease onset (HR 1.39, p = 0.005).

Conclusion: We found that missense mutations in NIPA1 do not play a major role in ALS. Instead, we found that short polyalanine expansions in NIPA1 are associated with ALS. In addition, these expansions are associated with an earlier disease onset and worse prognosis. These results further underscore the role of NIPA1 in ALS pathogenesis.

P09.011

CHRNA4 rare variants constitute a predisposing factor for sporadic ALS: results of a replication study in an Italian cohort of 682 patients

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Amyotrophic Lateral Sclerosis is a fatal neurodegenerative disorder, sporadic (sALS) in 95% of cases. Currently, multiple genetic and environmental factors are considered to contribute to disease liability. We previously observed that rare missense variants within the cytosolic loop of α3,α4,β4 subunits of neuronal nicotinic acetylcholine receptors (nAChRs) were over-represented in a group of 245 sALS patients compared with 450 controls.

Tested functionally in vitro, the observed variants were found to disrupt the receptor properties, leading to the hypothesis that they are a predisposing factor for sALS.

We made a replication study in new independent cohorts of 682 Italian sALS patients and 1300 age- and ethnically-matched controls, respectively. Sequencing analysis of *CHRNA4* gene, encoding for α4 subunit of nAChRs, revealed six heterozygous missense variants and a three-nucleotide insertion in 13 patients (1.9%). Four of the missense variants were detected in six controls, four were present in both groups (but were more numerous in patients), six were detected exclusively in controls. The three-nucleotide insertion has never been detected in controls. Using SIFT and PolyPhen, almost all variants found both in patients and controls or only in controls were predicted to be benign, while 50% of variants found exclusively in patients were predicted to be damaging. Combining present data with those reported previously, a total of 5 variants were detected only in patients but neither in 3500 chromosomes nor in dbSNP nor in 1000Genomes-Project data.

These data confirm our previous results, suggesting a possible role for *CHRNA4* variants as predisposing factor for sALS.

P09.012

Combined genome-wide analysis identifies *UNC13A* and chromosome 9p21.1 as shared loci for susceptibility to amyotrophic lateral sclerosis and frontotemporal lobar degeneration.

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Overlap exists for patients with Amyotrophic Lateral Sclerosis (ALS) and patients with frontotemporal lobar degeneration (FTLD), both clinically and pathologically as well as genetically. About 5-10% of ALS patients are diagnosed with FTLD, while FTLD patients can develop motor neuron symptoms. Furthermore, TDP-43 inclusions have been found in the majority of ALS patients and in cases of FTLD (FTLD-TDP). Previously, linkage studies in families with both ALS and FTLD have identified a locus on chromosome 9p13-21. We hypothesized that ALS and FTLD may be part of a spectrum of neurodegenerative disease and we sought to identify a common genetic basis for this neurodegenerative disease. We obtained genome-wide SNP genotype data from previously published studies on ALS and FTLD-TDP and performed genome-wide imputation based on the HapMap3r2 reference. After quality control, genome-wide data for ~1.2M SNPs were available for nearly 4,400 ALS patients and over 13,000 controls and for 440 (pathology-proven) FTLD-TDP cases and 1,400 independent unaffected controls. Joint analysis of ALS and FTLD identified SNPs in locus chromosome 9p21.1 and SNP rs12608932 in gene *UNC13A* as shared genetic variants contributing to susceptibility to both neurodegenerative disorders. Using a conservative rank-products analysis, weighing ALS and FTLD sample sizes equally, the associations were found to be consistent. By analyzing a subset of FTLD patients without motor neuron disease signs, we found that individuals with ALS symptoms did not solely drive the signal for both loci. Our study narrows the previously identified shared genetic locus on chromosome 9 and, additionally, implicates *UNC13A* as a shared risk locus further corroborating the role of *UNC13A* in neurodegeneration.

P09.013

UNC13A modifies susceptibility and survival in amyotrophic lateral sclerosis

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A large genome-wide screen in amyotrophic lateral sclerosis (ALS) showed that the common variant rs12608932 in gene UNC13A was associated with disease susceptibility. UNC13A regulates the release of neurotransmitters (including glutamate) by neuronal synaptic vesicles. Two attempts to replicate association with ALS susceptibility in small samples have not been successful. As a discovery cohort, we used genotype data from individuals of Dutch, Belgian or Swedish descent (1767 cases, 1817 controls) who had participated in a previously published genome-wide study of ALS and for whom survival data were available. An independent replication cohort of 450 ALS patients and 524 unaffected controls was recruited from a population-based study of ALS in The Netherlands. Using capillary sequencing we determined rs12608932 genotypes in the replication cohort. We

present an independent replication of association with rs12608932 (odds ratio 1.91, P = 0.01 for a recessive genetic model). Additionally, we show association of the minor allele of rs12608932 with shorter survival of ALS patients (genome-wide cohort: hazard ratio 1.22, P = 0.01; replication cohort: hazard ratio 1.62, P = 0.005 using a recessive model). ANOVA analyses showed that our data was best fitted with a recessive model. In our study we found a higher minor allele frequency in ALS cases compared to previous studies, while the minor allele frequency in controls was comparable. Our study shows the importance of patient selection methods in replicating genetic variants that affect both susceptibility and survival, as these characteristics can vary greatly between population-based and referral-based cohorts.

P09.014
Functional failure of neuromuscular junctions in transgenic *drosophila melanogaster* with human *APP* overexpression

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Although abnormal processing of amyloid-β precursor protein (APP) has been implicated in the pathogenic cascade leading to Alzheimer's disease (AD), the normal function of this protein and its role in synaptic dysfunction in AD is poorly understood. At the same time, deficits in synaptic plasticity and cognitive functions were detected in APP knockout or APP knockdown animal models. Moreover, independent evidences indicated that abnormal metabolism of APP (mutant APP or APP overexpression) might contribute to synaptic dysfunction independently from Aβ. We show previously that overexpression of full length human APP but not its truncated forms are sufficient for abnormal expression of synaptic proteins in the *Drosophila melanogaster* brain. To clarify APP function, we expressed wild-type APP and its mutant form *APP-Swedish* in larval motoneurons. We showed that APPs overexpression caused a dramatic functional disruption in the neuromuscular junction (NMJ). The NMJs exhibited abnormal endo/exocytosis that was determined by incorporation of the styryl dye FM2-64. Analysis of mitochondria distribution showed that motor neurons overexpressing *APP (APP-Swedish)* had a significant reduction of functional mitochondria in the presynaptic terminal. We propose that APP regulates synaptic structure and functions and its overexpression leads to synaptic pathology independently from neurotoxic effect of Aβ.

P09.015
Alzheimer's Disease: Causes, New risk Factors

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We still don't fully understand what causes Alzheimer's disease. What is clear is that Alzheimer's develops as a result of a complex cascade of biological processes that take place over many years inside the brain. But the majority of Alzheimer's cases are late-onset, usually developing after age 65, and this form of the disease shows no obvious inheritance pattern. However, in some families, clusters of cases are seen. The deposition of amyloid in the form of plaques is thought by many scientists to trigger the cascade of events leading to Alzheimer's pathology. Amyloid now is believed to be a critical target for eventual treatment. The best evidence that amyloid causes the disease comes from the genetic studies in which mutations of APP, PS1, PS2 and APOE e4 (the genes so far identified as causing some cases of Alzheimer's) all facilitate amyloid accumulation. Some current research is focused on the association between these two forms of ApoE and Alzheimer's disease. A growing body of research is also helping to identify various candidate genes. Several other genes also appear to influence the development of Alzheimer's disease: inflammatory pathways genes (TNF-α, CCR2, CCR5), calcium homeostasis modulator 1 (CALHM1), neuronal sortilinrelated receptor gene (SORL1). We examined a total of 160 samples prepared from patients diagnosed as being affected by Alzheimer's disease and 163 healthy controls from west northern Iran (Eastern Azerbaijan) for variants of these genes and genotype frequencies were statistically analyzed.

P09.016
Runs of Homozygosity and susceptibility to Alzheimer's disease

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Alzheimer's disease (AD) is the most common form of dementia; it is genetically complex and shows heritability up to 79%. Extended runs of homozygosity (ROHs) have been shown to occur at a high frequency in the human genome and may account for some of the unexplained genetic heritability of AD. Previous studies of ROHs in AD have failed to identify an overall excess burden of ROHs in sporadic late-onset AD cases, but one study did identify a ROH on chromosome 8 which was significantly overrepresented in cases when compared to controls. Using data generated from an AD genome-wide association study (GWAS) we describe here a statistical comparison of ROHs greater than 1Mb in a UK and Ireland series genotyped on the Illumina 610-quad array.

There were 1,955 AD cases, 955 elderly screened controls and 529,205 autosomal markers which were suitable for use in this study. ROHs analyses were performed using PLINK 1.07.

A total of 63,204 ROHs were identified. Burden analysis revealed no significant difference in the number of ROHs per person between cases and controls for ROHs greater than 1 Mb or when the data was stratified by size.

No region was significantly associated with disease after correction for multiple testing. The chromosome 8 region found to be associated with AD in a previous study did not show any association in this dataset.

In summary, ROHs are common in our outbred population, however statistically these ROHs are not a risk factor for late-onset AD in our dataset.

P09.017
Genetics of anal furunculosis in the German Shepherd Dog: Genome-wide association, replication, fine-mapping and comparative genomics.

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Anal furunculosis (AF) is characterised by ulceration and fistulation of perianal tissue and particularly affects German Shepherd Dogs (GSD). It has drawn parallels with perianal Crohn's disease in humans. An immune-mediated aetiopathology has been suggested due to a strong MHC association and clinical response to ciclosporin. A Genome-wide association study (22,362 SNPs) was performed on 21 affected and 25 unaffected GSDs. Areas of potential interest were followed-up by genotyping 91 SNPs in a further 54 cases and 110 controls. Three loci were significantly associated after multiple testing had been taken into account. A good candidate gene region on chromosome 34 was discovered, in which there were five protective SNP associations (p = 0.018 - 0.029, OR = 0.391 - 0.466). Additionally, we have identified a region of extended homozygosity (selective sweep) on chromosome 3 associated with this disease. Targeted sequencing of these regions is currently underway. As this canine disease is of comparative interest to human medicine, an analysis was carried out using Crohn's disease patients: 456 with perianal disease and 1197 without perianal complications. We selected 34 regions containing 42 SNPs showing

some association with AF from the follow-up study which could be syntenically mapped to the human genome. When investigating 1Mb regions around these SNPs in the perianal Crohn's disease cohort, there were 25 SNP associations having a raw p value < 0.05, including one upstream of the canine candidate gene (p = 0.023). This suggests a possible common genetic component to both these canine and human conditions.

P09.018**

A long non-coding RNA accounts for Angelman syndrome-like neurodevelopmental disorder

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Angelman syndrome (AS) is a neurodevelopmental disorder characterized by severe mental retardation and autistic-like features. AS is due to lack of UBE3A gene expression, but in 15% of the cases the molecular cause still remains unknown. Long non-coding RNAs (lncRNA) constitute a large portion of the human transcriptome, which regulates gene expression by different mechanisms, including splicing, translational repression and epigenetic regulation. The spatial and temporal expression of lncRNA appears to be extremely important for the development and function of the central nervous system. Our study is focused on an AS-like patient with an unusual combination of a deletion that involves lncRNA AK097590 inherited from one parent, and a rare SNP within AK097590 inherited from the other parent. Besides, this SNP leads to a change in the predicted secondary structure of AK097590 that may affect its normal function. We have performed AK097590 knock down experiments in differentiated human neuroblastoma cells. qPCR assays have demonstrated that a decrease of AK097590 levels is sufficient to modify the expression of UBE3A, PRKCG and PPP2R1A. Interestingly, these genes have a role in synaptic plasticity and long-term depression, which are reduced in AS murine models. Our results suggest that changes in the expression levels of AK097590 may contribute to the clinical features associated to AS, offering a novel molecular basis for the cases non associated with mutations in UBE3A. A better understanding of the regulatory mechanism of AK097590 lncRNA and its target genes may help in the development of therapeutic strategies for these neurodevelopmental disorders.

P09.019

TNFalpha gene polymorphisms in Romanian HLA-B27-positive patients with ankylosing spondylitis

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Background: Ankylosing spondylitis (AS), the prototypic seronegative spondyloarthritis is a chronic inflammatory condition of the spine and sacroiliac joints. HLA-B27 is a genetic marker for AS. Moreover, elucidating the role of tumor necrosis factor alpha (TNFalpha) in AS has important diagnostic and therapeutic significance, given the good results obtained with anti-TNF therapy in these patients.
Objectives: The aim of this study was to investigate three TNFalpha gene single nucleotide polymorphisms (SNPs) in HLA-B27-positive ankylosing spondylitis patients from Romania.
Methods: 112 Romanian unrelated AS patients (97M/15F) and 76 (40M/36F) ethnically match healthy controls were genotyped for TNF-alpha -308G/A, -238G/A and -857C/T polymorphisms using TaqMan SNP Genotyping Assays C-7514879-10, C-2215707-10 and C- 11918223-10 (Applied Biosystems, USA). All subjects were HLA-B27 positives. Association tests for each polymorphism and for the estimated haplotypes were performed with the software package PLINK v 1.07.
Results: The minor allele TNF-857T was more frequent in AS patients (24%) than in controls (19%), but not statistically significant (p=0.2). None of the three SNPs were individually associated with susceptibility to AS in HLA-B27-positive patients.
Two haplotypes showed a negative association with the disease: 308G238A (p=0.04) and 857T238A (p=0.005).
Conclusion: These data indicate that haplotypic variations in the TNFalpha gene influence disease susceptibility in HLA-B27 positive individuals in our population. The protective effect of these haplotypes could be related to differences in TNFalpha production or could reflect the association of different HLA-B27 haplotypes with ankylosing spondylitis.

P09.020

Replication study of genome-wide discovered loci associated with anti-tumour necrosis factor treatment outcome in patients with rheumatoid arthritis. Results from the DREAM registry.

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Agents blocking tumour necrosis factor (anti-TNF) are widely used for the treatment of rheumatoid arthritis (RA). Unfortunately, ~30% of the patients do not respond to this treatment. Although there is some indication that genetic variants may influence the response to anti-TNF none of these variants can reliably predict treatment outcome. We aimed to replicate findings from three published genome-wide studies. RA patients treated with anti-TNF from the Dutch Rheumatoid Arthritis Monitoring (DREAM) registry were included. Single Nucleotide Polymorphisms (SNPs) located within 50 kb of SNPs previously found associated with anti-TNF outcome were extracted from genome-wide data (Illumina HumanHap550-Duo/Human660W-Quad BeadChips). Single-SNP and gene-based association analysis were performed in PLINK using the percentage disease activity score (DAS28) change at 3 (n=503) and 6 months (n=386) as outcome.
16 of the 24 SNPs included were located near or within the genes *GBP6, LASS6, AKO93144, PON1, MOBKL2B, C9orf72, TEC, PDZD2, EYA4, BC118985* and *PTPRC.* The other SNPs were located in gene-desserts on chromosome 1p22.3, 1q24.1, 4p15.1,11p14.2, 12p12.3 and 20p11.21. Only the single SNP analysis at six months revealed significant findings. Three SNPs, located in *GBP6* (p=0.016), which is regulated by inflammatory cytokines, locus 20p11.21 (p=0.006) and PDZD2 playing a role in insulin secretion related to disease severity in early RA patients (p=0.045), were associated with treatment outcome. All associations lost significance after multiple-testing correction.
Three previously reported loci show suggestive association with anti-TNF response in the Dutch population. Larger datasets are required to determine whether these genetic variants are indeed associated with anti-TNF outcome.

P09.021

Association study between telomere length and Age-Related Hearing Impairment in 3527 Caucasian individuals and 663 Japanese individuals.

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It has been proposed that individuals with shorter telomere length are more susceptible to age-related disorders. In somatic cells, telomere length declines with age contributing to loss of cells with age when telomeres reach a critical minimal length. Average telomere length was shown to be a heritable trait (Slagboom et al., 1994).
Age-Related Hearing Impairment (ARHI) is the most common sensory disorder and the most important cause of hearing loss in the elderly, with a negative impact on the quality of life. It is a complex disease influenced by genetic as well as environmental factors. Here we test

whether a shorter average telomere length leads to increase of ARHI susceptibility. In 3527 European and 663 Japanese individuals, we determined the telomere length in white blood cells using a monochrome multiplex quantitative PCR protocol (described by Cawthon, 2009). We performed association testing between the telomere length and the 3 Principal Components of the ARHI phenotype (Huyghe et al., 2008). In addition, we tested this association adjusting for environmental factors that were previously implicated in telomere shortening. The most important of these factors include smoking and BMI.

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P09.022

Association analysis of *Dysbindin* polymorphisms P1635 and P1655 with Schizophrenia

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Schizophrenia is a severe psychiatric disorder that has a lifetime prevalence of ~1% in most of the population studied. Similar to many common complex disorders, schizophrenia is a multifactorial disorder that is characterized by the contribution of multiple susceptibility genes that could act in conjunction with epigenetic processes and environmental factors. There is quite impressive evidence supporting the association between genetic variants in dysbindin and schizophrenia in populations. In the present study, we investigated the association between polymorphisms P1635 and P1655 in dysbindin gene with schizophrenia. Subjects were 115 unrelated patients with schizophrenia and 117 unrelated healthy volunteers. Genome-DNA was extracted from blood sample by using the standard salting out method. Genotyping was done with the PCR-RFLP method. The allele and genotype association were analyzed with χ^2 test. As the result, no significant difference was observed between patients and controls in allelic frequencies or genotypic distributions of the P1635 SNP (p=0.809), but we could find a significant difference between case and controls for the P1655 SNP (p=0.009). **).** Also we could find a significant association in A-C haplotype in patient with SCZ (p=0.004, OR= 1.7) and a protective effect in A-G haplotype (p= 0.003, OR=0.57). The present study may provide further support for an association between P1655 SNP and schizophrenia in Iranian populations. Studies with more markers and subjects for various populations will be necessary to understand the genetic contribution of Dysbindin gene for the development of schizophrenia.

P09.023

Association analysis of polymorphisms in selected genes in Slovenian children with asthma

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Many candidate genes initially associated with asthma or asthma phenotypes were not confirmed as asthma genes in the follow up independent studies in different populations. In the most association studies candidate genes were compared between controls and all asthma patients in one group. The aim of our study was the association analysis of selected genes in Slovenian asthmatic children. We analyzed candidate genes in all asthmatics and separately in the group of atopic asthmatics and the group of non-atopic asthmatics. In addition, we have analyzed the correlation among selected polymorphisms, clinical parameters and response to therapy with glucocorticoids. Using bioinformatics tools nine single nucleotide polymorphisms (SNPs) in nine different candidate genes were selected. We have genotyped 304 children with asthma and 195 healthy controls. We have performed genotyping by polymerase chain reaction (PCR) and by the restriction fragment length polymorphism (RFLP) analysis. We have found significant association of genes CCR5, IL13 and RANTES and asthma. Our study was the first that associated IL12B and IL13 gene with a nitrogen in the exhaled air of asthmatic patients. In addition we were the first ones who associated IL13 and ORMDL3 with response to asthma therapy with glucocorticoids in the group of atopic asthmatics. Our results contribute to our understanding of the asthma pathogenesis. Identification of polymorphisms associated with asthma phenotypes and response to therapy may lead custom tailored and better management of asthma patients.

P09.024

Assessing the reproducibility of eight asthma candidate gene associations in the Spanish population

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Before the advent of genome-wide association studies (GWAS), a massive number of independent candidate genes studies suggested that SNPs from *ADAM33, ADRB2, CD14, MS4A2* (alias *FCER1B*), *IL13, IL4, IL4R,* and *LTA-TNF* genes were among the most replicated associations with asthma. To date, except for the *IL13-IL4* region, none of these genes was found in close proximity of GWAS hits in asthma or related traits. Among studies with Spanish samples, most have comprised direct associations of 1-2 SNPs/gene and limited case numbers. To test the credibility of associations of these eight candidate genes with asthma, we systematically evaluated previously associated SNPs as well as tagging SNPs (average r^2=0.96) in a large study sample of 606 asthmatic cases from the Genetics Of Asthma (GOA) study and 1259 population-based controls. After population stratification adjustments based on 83 additional SNPs, we found nominal significant associations with asthma for 14 SNPs in 4 genes (*IL4R*, *ADAM33, MS4A2,* and *LTA-TNF;* 0.004≤p-value≤0.047), including some of the previously associated SNPs (*MS4A2*: Gly237Glu; *IL4R*: Gln551Arg, Cys406Arg and Ser478Pro; *LTA-TNF*: -857C/T and -753G/A). Despite no associations would remain significant after adjusting for the multiple comparisons performed, these findings suggest that *IL4R*, *ADAM33, MS4A2,* and *LTA-TNF* genes should be more exhaustively analyzed for putative causal variants.

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P09.025

The association between 17q12-21 SNPs and asthma in the Volga-Ural region of Russia.

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Asthma is one of the most common multifactor diseases, in which many cells and cellular elements play a role. The first GWAS for asthma discovered a significant association between 17q12-21 SNPs and childhood asthma in Europeans. Consistently, its results have been successfully replicated in independent populations of European and Asian ancestries. The aim of our investigation was to identify asthma susceptibility genes in the Volga-Ural region of Russia. The study included 358 unrelated patients with physician-diagnosed asthma and 369 disease-free control subjects of different ethnic origin (Russians, Tatars and Bashkirs). The genotyping was carried out using the Illumina Human610 quad array at the CNG (France) as a part of GABRIEL (A Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community). Statistical tests for association were carried out using PLINK 1.06 software package. In order to control for population stratification, we performed Eigenstrat analysis for cases and controls, removing outliers. After QC filters,

550915 SNPs genotyped in 330 cases and 348 controls were used for testing the association of SNPs to asthma. Some markers on chromosome 17q12-21 within the ORMDL3/GSDMB locus showed the most statistically significant association in this study ($p \leq 5x10^{-7}$). The maximum association was at the SNP rs7216389 within 1 intron of the GSDMB gene ($p=1.013x10^{-7}$). In summary, we have found the association asthma in the Volga-Ural region of Russia with SNPs at the previously reported ORMDL3/GSDMB locus on 17q12-21. Supported by a contract from the EC (018996).

P09.026

Genotyping of *ATG16L1* rs2241879 and IL23R rs1004819 polymorphisms among Polish patients with Crohn's disease and localization of symptoms in gastrointestinal tract

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Introduction: Crohn's disease (CD), next to ulcerative colitis (UC), belongs to Inflammatory Bowel Diseases (IBD). Genes *ATG16L1* and *IL23R* may predispose for the development of the disease.

Aim: of the research was the analysis of polymorphisms: *ATG16L1* rs2241879 and IL23R rs1004819 among Polish CD patients. We also wanted to find out if any of these two polymorphisms is related to localization of GIT symptoms.

Materials and methods: 96 Polish patients with CD and 96 individuals from population group were qualified for research. Method used for genotyping was pyrosequencing. Statistical analysis was performed.

Results: Analysis of ATGrs2241879 revealed that the most frequent was GA genotype (55,7% among CD patients, 48,7% in population group), next AA (27,1% and 31,2%, respectively) and GG (17,1% and 20,1%). Analysis of IL23Rrs 1004819 revealed that TC genotyope was the most frequent among CD patients - 51,1% (35,4% in population group), while in population group the most frequent was CC genotype (48,7%, and 44,7% among CD patients). TT genotype was observed in 4,3% of CD patients and 15,9% of population group. Alleles analysis didn't reveal any statisticaly significant differences in frequency of genotypes and alleles. Statistical analysis revealed that the most frequent genotype in ATGrs2241879 among patients with intestinal manifestation of the disease was GA, while in IL23R rs1004819 it was TT genotype($p=0.005$).

Conclusions: Although we did not find any statistically significant differences in genotypes frequency among CD patients and population group, we observed some tendencies related to intestinal localization of symptoms and specific genotypes.

P09.027

No increased risk for atherosclerosis in migraine patients from the ERF study

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Background and Aim: Migraine, in particular migraine with aura, is associated with an increased risk of ischemic stroke and coronary heart disease. It is unknown whether this is mediated by an unfavorable cardiovascular risk profile and associated atherosclerosis. Therefore, we investigated functional, structural, and genetic markers of atherosclerosis and cardiovascular disease in migraine patients.

Methods: Subjects were participants of the Erasmus Rucphen Family (ERF) genetic isolate study, in which confounding is reduced because of the relatively homogenous genetic and environmental background of subjects. Atherosclerosis was assessed in 360 migraineurs (209 had migraine without aura and 151 had migraine with aura) and 617 subjects without migraine or severe headache. Atherosclerosis was quantified by Intima Media Thickness (IMT), Pulse Wave Velocity (PWV) and Ankle-Brachial Index (ABI). A large number of putative cardiovascular risk parameters and the Framingham risk scores for coronary heart disease (CHD-score) and stroke (stroke-score) were evaluated and gene-based p-values were calculated for two genes implicated in cardiovascular disease (*MTHFR* and *ACE*).

Results: No difference was found in carotid IMT, mean PWV, or ABI between migraine patients and controls. Migraine patients had no increased odds of having an elevated CHD-score or elevated stroke-score (>10%). No association with *MTHFR* or *ACE* was found.

Conclusions: Migraineurs do not have increased risk for central or peripheral atherosclerosis. Our data suggest that traditional cardiovascular risk factors and atherosclerosis are an unlikely explanation for the previously observed association between migraine and cardiovascular disease.

P09.028

Common genetic variants in *ITGA2*, *IL1B*, *ALOX5AP*, *OR13G1*, *MMP9* genes that participate in oxidative stress and influence atherosclerosis phenotype formation

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Atherosclerosis is complex multifactorial disorder where both genetic and environmental factors play an essential role in the etiopathogenesis of the disease. Formation and further progression of atherosclerosis is based on the intricate biological pathways. Our previous study (Linkoping-Vilnius coronary disease risk assessment study) showed the higher mortality from coronary heart disease (CHD) of Lithuanian men in comparison with Swedish men. Subsequent observations revealed that Lithuanian men had some differences in atherosclerosis phenotype, which may be influenced by complementary factors. It appears that one of these factors could be the oxidative stress. Thus the goal of this study was to evaluate the mainstream biological pathways inducing and maintaining the atherosclerotic process by analyzing genetic biomarkers particularly in inflammatory, metabolic pathways where the main focus is laid on oxidation process. After bioinformatic analysis (Medline data mining) there were 150 SNPs in 89 genes selected for the construction of microarray based on the APEX technology (Asper Biotech, Estonia). Genotyping was carried out in 23 families and the TDT, S-TDT as well as combined analysis were performed. The following SNPs associated with atherosclerosis phenotype were identified: rs1126643, rs1143627, rs9551963, rs1151640, rs3918242 in the candidate genes *ITGA2*, *IL1B*, *ALOX5AP*, *OR13G1*, *MMP9* respectively. These genes belong to different biological pathways: trombocyte adhesion and vessel damage (*ITGA2*, *MMP9*), inflammation response (*IL1B*), cholesterol and lypoxygenase metabolic pathway (*ALOX5AP*) and nutrition (*OR13G1*). Generalized biochemical, bioinformatical and candidate genes' association results seem to support hypothesis that oxidation process may be of key importance in atherosclerosis formation.

P09.029

A comparative analysis of SNPs of ADRB1 gene in children with a bronchial asthma and in healthy children

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Background: The majority of undesirable effects associated with regular use of β2-agonists in patients with the atopic bronchial asthma (BA) are connected with their action on β1-adrenoretseptor. In modern pharmacogenetics, studies on SNPs of human β1-adrenoretseptor gene (ADRB1) become topical.

Study groups: The study group included 272 children with BA (47 girls

and 225 boys) aged from 4 to 17 years old. The control group included 134 healthy children (64 girls and 70 boys) aged from 6 to 17 years old.
Methods: The genetic polymorphism of ADRB1 gene was identified by PCR-RFLP standard method.
Results: The distributions of genotypes and alleles of the SNPs in the ADRB1 gene were not significantly different between studied groups. However, we have found a significant difference between patients with various variants of BA. The patients with mild asthma had a combination of genotypes S49G/G389G [4,5% vs 0,0%] and G49G/R389R [4,5% vs 1,9%] more frequently compared to those suffered from severe asthma in whom S49S/G389G [6,7% vs 3,0%] and S49G/R389R [20,0% vs 9,1%] prevailed (p<0,05).
Conclusion: These results suggest that polymorphisms of the ADRB1 gene might play a role in severity of asthma manifestation, and that ADRB1 polymorphisms is not associated with BA in Russian population.

P09.030
German shepherds as a canine model for Atopic dermatitis

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Canine Atopic Dermatitis (CAD) is a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features. It is most commonly associated with IgE antibodies directed towards environmental allergens, often house dust mites. Onset of clinical signs is usually between 6 months and 3 years of age. Initial signs can be seasonal or non-seasonal depending on allergens involved. In a similar pattern as in human atopic dermatitis typically face, ears, paws, extremities, ventrum and flex-zones are affected. The diagnosis is based on clinical signs and exclusion of differential diagnoses, such as flea allergy dermatitis, cutaneous adverse food reactions, scabies or other pruritic ectoparasites, pruritic bacterial folliculitis and *Malassezia* dermatitis. A positive allergen-specific IgE test (serology or intradermal test) aids in defining offending allergens. Some breeds have markedly higher prevalence of CAD compared to others. German shepherds have an exceptionally high susceptibility to many immune-related diseases, including CAD.
We have performed a genome-wide association study of 91 German shepherd CAD cases and 93 controls using the Illumina ~170 K canine SNP array. In addition, IgA levels were measured in serum from the same German shepherd dogs.. In concordance with other studies, the German shepherds showed lower IgA levels compared to other breeds. Further, the IgA levels were significantly lower in German shepherd CAD cases compared to controls (p=0.000008). Thus, IgA levels were included as a covariate in genome-wide association analysis using GenABEL. Four regions with clear association are currently being investigated further in both canine and human cases and controls.

P09.031
Association analysis of cytokine gene polymorphisms and atopic dermatitis in Volga-Ural region of Russia.

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Atopic dermatitis (AD) is a chronic inflammatory skin disease associated with cutaneous hyperreactivity to environmental triggers and is often the first step in the atopic march. Cytokines play crucial role in all stages of allergy and inflammation development and maintaining. Significant associations between single nucleotide polymorphisms (SNPs) in cytokines and cytokines receptors with AD have been replicated in more than one study. In this study we have screened SNPs in the IL4 (-590C>T), IL4RA (Ile50Val), IL13 (Arg130Gln) and CCL11 (−384A>G) genes in AD patients and control individuals. The AD group consisted of 214 AD patients with different ethnic origins (88 Russians, 44 Tatars and 82 individuals of mixed origins). The control group included 200 non-atopic individuals (55 Russians, 49 Tatars and 84 individuals of mixed origins). Genomic DNA was isolated by phenol-chloroform extraction. The genotyping of SNPs was performed by PCR-RFLP.
Significant differences between AD patients and control individuals of Tatar ethnic origin were found in the allelic frequencies for the Ile50Val polymorphism of the IL4R gene. The frequency of the allele Val in AD patients was significantly higher than in control group - 0.466 versus 0.327, respectively ($\chi 2=3.78$, p=0.05). The estimated odds ratio for the allele Val at this SNP was 1,8 (CI95% 0,99-3,26), for the allele Ile - 0.56 (95%CI 0.31-1.0). The analysis of -590C>T polymorphism of the IL4 gene, Arg130Gln polymorphism of the IL13 gene and −384A>G polymorphism of the CCL11 gene has detected that there are no significant differences between AD patients and healthy donors.

P09.032***
Association of the ciliary gene *AHI1* with autism

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Joubert syndrome (JBTS) is an autosomaal recessive disorder characterized by hypoplasia of the cerebellar vermis, the characteristic neuroradiologic 'molar tooth sign,' and neurologic symptoms, including dysregulation of breathing and developmental delay. JBTS also includes variable features such as retinal dystrophy and renal anomalies. Up to 27% of JBTS patients show features of autism. Thus far, 10 genes for JBTS have been identified. The ciliary gene *AHI1* has been found mutated in 7 to 16% of cases with JBTS. On the other hand, of the 39 genes associated with autism, 29 (74%) encode ciliary genes. This association of ciliary genes with autism is highly significant ($P < 0.001$; two sided Fischer exact test). Therefore, we tested the hypothesis that ciliary genes may be involved in autism, by performing a candidate-gene association study with a cohort of 84 patients with autism vs. 145 healthy controls for the 10 known JBTS genes (*AHI1, NPHP1, CEP290, ARL13B, RPGRIP1L, MKS3, CC2D2A, OFD1, TMEM216,* and *INPP5E*). For *AHI1* we found nominal association with odds ratio's from 3.077 to 9.174 and p values for association < 0.0001 for SNPs rs12179084, rs2327587 and rs7766656. Our data suggest that dysfunction of primary cilia may constitute an important neuropathological pathway in autism.

P09.033
aCGH analysis of two families showing both autism and epilepsy

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Autism is a genetic disorder, with an estimated heritability greater than 90%. Over the past years, the convergence of genomic technologies has led to the identification of several susceptibility loci by means of linkage studies. Autism is associated with epilepsy in early childhood and epilepsy occurs in 10-30% of autism patients.
We analysed two families showing both autism and epilepsy. The first one is the family of a woman, showing a severe and progressive epileptic disorder, who is married with an healthy man and has six children: two girls are healthy, a girl and two boys are affected by autism, while a boy showed partial seizures only twice. The three children with autism show a moderate mental retardation and an EEG with no epileptic alterations. The second family is composed by an epileptic woman married with a healthy man and their child affected by autism.The genetic basis of autism and epilepsy were studied in these families. In particular, we performed an array-CGH analysis to search for pathological CNVs which are common to all affected members of the two families.
The study detected a single common region on chromosome 15q11.2, which has already been reported to be either deleted or duplicated in about 1% of epileptic patients. Moreover, the same region was associated with ASDs (autism spectrum disorders). We found a loss of 15q11.2 in all individuals belonging to the first family, while a gain of the region in the two affected members of the second family.

P09.034

Prevalence of fragile X syndrome in non-syndromic autistic children in western saudi arabia

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Prevalence of Fragile X Syndrome (FXS) in the general population is around 1 in 1000 males and 1 in 2500 females. It is widely reported in the literature that FXS in the autistic populati5%, and that 30% of the FXS cases have full Autism. Among the remaining patients with FXS, of those who do not meet the criteria for an autism spectrum disorder (ASD) diagnosis, the majority have one or more autistic features, such as hand flapping, poor eye contact and tactile defensiveness. Also it is reported that FXS is the most common single gene cause of autism, responsible for 2% to 6% of all cases of autism.

We studied 41 patients with non-syndromic Autism in Western Saudi Arabia, we determined the trinucleotide repeat number (TRN) of CGG in 5' end for the untranslated region of the gene FMR1 using PCR technique as described by Chong et al, (AJMG 51:522-526, 1994).

After performing gel electrophoresis for the PCR products using 2% agarose gel, the results analysis demonstrated that all samples have a CGG trinucleotide repeat number less than 50 repeats.

In conclusion even though our sample number is small, with the percentage reported FMR1 gene mutation seems to not play a major role in the etiology of Autism in Western Saudi Arabia. There is a much lower prevalence of FXS. We may attribute this to a larger role of recessive causes, especially if considering parental consanguinity, though there may be other reasons as well.

P09.035

Characterisation of putative autism susceptibility genes: Translating genome wide analysis to causation

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Introduction

Genomewide Association studies (GWAS) have been used to identify genetic markers which have strong statistical association with a disease. Establishing a functional consequence of association is required to determine a causal link between marker and disease. In a recent GWA of autism we identified a strong association within the gene MACROD2 (1). Very little is known about the function of MACROD2. This project is structured to examine the biological role of MACROD2, with the hypothesis that disruption of MACROD2 will impact on the structure and/or function of the neuron.

Results

MACROD2 is expressed almost ubiquitously across human tissues, including brain, kidney, placenta, skeletal muscle, testis and thyroid gland. Promoter mapping constructs were examined in SHSY5Y neuroblastoma cell lines. We observed differential expression of the various promoter lengths. From this preliminary data it would appear that the promoter region of MACROD2 lies ~500bp upstream from exon 1.

Discussion

GWA offer potential insight into the biology of disease. The biological characterisation of observed candidate genes must follow GWA to best take advantage of these data.

This preliminary work is part of a wider comprehensive study of MACROD2 which will include both in silico and in vitro techniques.

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P09.036**

Large-scale genetic pathway analysis of copy number variants highlights important rare genetic causes and gene networks for autism spectrum disorders

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The ability to quantify individual's genomic risk for disease can facilitate the development of new interventions and improve medical practice. Many rare Copy Number Variants (CNVs) that harbor small genomic deletions and insertions have been described in the autism spectrum disorders (ASD). To identify these likely functional elements, we combined three previously published large cohorts of autistic patients with a large number of controls to analyze over 25K unrelated individuals. After stringent quality control measures, we compared 3K cases to over 19K ethnically matched controls in a two-stage genome-wide association design. In all, we uncovered 275 statistically significant distinct copy number variable regions. The 60 genes nearest these robust CNVRs are most enriched in gene networks impacting neurological disease, behavior and developmental disorder. A more focused analysis of a subset of 87 CNVRs that are predicted to disrupt 26 genes highlights GABA receptor signaling, and methionine/glutathione trans-sulfuration as the most significant canonical pathways disrupted in ASD. Furthermore, we used available family members to characterize CNVRs into inherited vs de-novo and used ethnicity information to characterize their effects in different ethnic populations. Taken together, the CNVRs we have identified impact multiple novel genes and signaling pathways, including genes involved in GABA receptor signaling, that may be important for new personalized therapeutic development.

P09.037

Identification of four mutations affecting normal splicing during the resequencing of X-chromosome synaptic genes in individuals with autism or schizophrenia

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Autism (AUT) and schizophrenia (SCZ) are two common neurodevelopmental disorders, which result from the combination of genetic and environmental factors. To explore the hypothesis that rare highly penetrant mutations in different genes specific to single families are involved in these diseases, we have resequenced X-linked synaptic genes in a cohort of 285 AUT and SCZ individuals and identified truncating mutations in IL1RAPL1 and MAOB genes (Piton A, Mol. Psy., 2010). We wanted to evaluate if, besides these two mutations, some of the rare variants could have such a drastic effect at the protein level by affecting the mRNA splicing. We used an in silico approach (using Human Splicing Finder, Nnsplice, SpliceView, ESEfinder, Rescue ESE) to detect which of the rare variants could create/disrupt an acceptor site, a donor site or an exonic splicing enhancer (ESE). Fourteen variants with the best prediction scores were tested in vitro using a minigene system in the vector SPL3B. We showed that four of these variants (28%), in GABRQ, GABRA3, HDAC6 and NXF5 genes, affect the normal splicing and are predicted to lead to truncated proteins. Our results illustrate the importance of analyzing the effect of mutations at the mRNA level and have led to the identification of new genes associated with AUT. Interestingly, although it has been considered since a long time that impairments in GABA pathways play a role in AUT etiology, it is the first time that truncating mutations in GABA receptors genes are identified in patients.

P09.038

Gene ontology enrichment analysis in two independent family-based samples of over 2100 families highlights biologically plausible processes for autism spectrum disorders

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There is growing evidence to support the role of rare structural and sequence variation in the aetiology of autism spectrum disorders (ASDs). However, genome-wide association studies (GWAS) of ASDs have yielded only modest evidence to support common variation in the aetiology of disorder. GWAS rely upon statistically robust genome-wide-significant associations to identify genes and loci of risk. There are considerable modest association signals that do not meet stringent genome-wide-significance thresholds. These signals may conceal underlying patterns that are important to understanding the aetiology

of disease. We have developed a robust method, pedSNP-ratio-test (pedSRT) to examine association enrichment in biologically-linked gene-sets for family-based association studies. Using a two-stage approach we explore association enrichment in GWA data from more than 2100 families from the Autism Genome Project. Based on estimates from study-sensitive simulation we identify excess of observed and replicated association enrichment. We highlight enrichment for sets of genes involved in diverse biological processes including pyruvate metabolism, transcription factor activation and cell-signalling. Many of the genes and processes that show enrichment have previously been examined in autistic disorders and offer biologically plausibility to these findings. Moreover, our data supports the hypothesis, that in part, common variation is important in the aetiology of ASDs. This work is presented on behalf of the Autism Genome Project Consortium.

P09.039**

AKAPs integrate genome-wide association findings for autism spectrum disorders into signalling networks regulating steroidogenesis, neurite outgrowth and synaptic function

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Background: Autism spectrum disorders (ASD) are highly heritable. In recent years, several genome-wide association studies (GWAS) of ASD were published.

Methods: We have carried out a bioinformatics analysis using Ingenuity and a systematic literature analysis of 200 genes from five published GWAS that yielded association with ASD through single nucleotide polymorphisms at $P < 0.0001$, and we used the results from a sixth published GWAS to validate our findings. We also searched for overlap between the identified candidate genes and genes located in copy number variations (CNVs) in people with ASD and we identified microRNAs that downregulate gene expression of ASD candidate genes and are deregulated in ASD.

Results: A total of 120 of the 200 ASD candidate genes encode proteins that fit into three signalling networks regulating steroidogenesis, neurite outgrowth and synaptic function, and we were able to place 50 other ASD candidate genes in the identified networks. The proteins encoded by 11 ASD-linked AKAP (A-kinase anchor protein) genes functionally integrate signalling cascades within and between these networks. Lastly, we found that 70 of the 200 ASD candidate genes were found in CNVs in people with ASD and 100 of these genes were downregulated by ASD-implicated microRNAs.

Conclusions

We have identified three signalling networks for ASD that contribute to our understanding of the molecular basis of the disorder and that are functionally integrated by the AKAPs, 'druggable' proteins that need to be further investigated as possible targets for psychopharmacological treatments of ASD.

P09.040

A novel unstable duplication upstream of HAS2 in Chinese Shar-Pei dogs causes the breed defining skin phenotype and a disease analogous to human periodic fever syndromes.

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The dog provides us with a spontaneous mutational model for disease. By exploiting the unique genetic signatures underpinning domestication and breed formation we can use genome-wide association to identify disease genes, which may also have roles in human disease. Autoinflammatory diseases have no known pathogenic or autoimmune cause and are characterised by recurrent episodes of fever and inflammation. Several genes have been implicated in human disease, however the majority of cases remain unexplained. The Shar-Pei breed suffers a similar autoinflammatory disease and has coincidently undergone selection for a distinctive wrinkled skin. Using genome-wide SNP analyses, the strongest signal of a breed-specific selective sweep co-localized with the fever susceptibility locus on chromosome 13. Re-sequencing identified two partially overlapping duplications, which were unique to Shar-Pei and located upstream of Hyaluronic Acid Synthase 2 (HAS2). HAS2 encodes the rate-limiting enzyme synthesizing hyaluronan (HA), a major component of the skin. HAS2 is up-regulated and HA accumulates in the skin of Shar-Pei. A high copy number of the 16.1kb duplication was associated with increased HAS2 expression and disease ($p < 0.0001$). Fragmented HA can act as a trigger of the innate immune system and stimulate sterile fever and inflammation. The strong selection for the skin phenotype appears to enrich for a pleiotropic mutation predisposing these dogs to disease. HAS2 was not previously associated with autoinflammation and preliminary results indicate that this glycosaminoglycan may be involved in human periodic fevers. Further inroads into the significance of this pathway for disease will be discussed.

P09.041

Genetic and epigenetic alterations in patients with Balkan endemic nephropathy

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BACKGROUND: Balkan endemic nephropathy (BEN) represents a chronic progressive interstitial nephritis in striking correlation with uroepithelial tumors of the upper urinary tract. The disease has endemic distribution in the Danube river regions in several Balkan countries. The so far conducted investigations of environmental factors as an ethological factor proved to be futile. The family and genetic studies were inconclusive.

Epigenetic changes encompass an array of molecular modifications to both DNA and chromatin. Epigenetic tests can elucidate the intricate pathogenetic mechanisms of BEN and can prove to be the bridge between environmental factors and genetic background in BEN development.

MATERIALS AND METHODS: We've collected blood and tumor samples. We've adopted genome-wide strategy (methylated DNA immunoprecipitation (MeDIP) and whole-genome array analysis. We will perform pool analysis to identify significant methylation status differences by comparing four cohorts: (1) affected by BEN individuals with at least one affected parent; (2) healthy individuals from endemic regions with at least one affected parent; (3) healthy controls form endemic regions with no BEN family history; (4) healthy controls from non-endemic regions.

RESULTS: Our previous experiments showed that in BEN patients the 3q25 band was most frequently involved in chromosomal aberrations. In superficial (pTa) BEN tumor the genome instability was extremely high. Comparative Genomic Hybridization (CGH) showed genetic gains at 1q, 3q, 7p, 7q, 15q, and 19q in BEN tumors analysed.

CONCLUSIONS: The data gleaned from own experiments have disclosed a strong impact of genetic factors to the development of BEN and BEN-associated urothelial tumors.

P09.042
Modifying effect of the *FTO* genetic variant on longitudinal association between adolescent emotional problems and adult body mass index

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Adolescent emotional problems have a long-tern effect on BMI change and obesity. Variations of *FTO* gene contribute to common forms of human obesity, and to BMI change across the life course. No study has investigated the joint effect of the *FTO* risk variant and emotional problems on BMI change across the life course. We aimed to investigate whether the effect of emotional problems on BMI change through adulthood is modified by the polymorphism of *FTO* gene.

We used data from a British 1946 birth cohort. We analyzed *FTO* rs9939609 (1238 men and 1233 women), which was previously associated with BMI in this cohort, in those with adolescent emotional problems and in those without. We tested for the interaction effect between *FTO* and affective status on BMI at ages 20, 26, 36, 43 and 53 years and on BMI change over a period of 33 years using multilevel models.

We showed that emotional problems were associated with lower BMI at age 20 (p=0.009), 26 (p=0.02) and 36 (p=0.005) years in *FTO* A allele carriers, but not in TT homozygotes. A significant interaction between *FTO* and affective status in relation to BMI was observed at age 20 (p=0.055) and 36 (p=0.008). Among A allele carriers, but not among TT homozygotes, those with adolescent emotional problems had faster rates of BMI increase across adulthood.

The relationship between emotional problems and BMI varies by *FTO* genotype. This finding has implications for predicting which people with emotional problems are at higher risk of weight gain.

P09.043
Genetic variation of glutamate-cysteine ligase modifier subunit and susceptibility to nonallergic bronchial asthma: an evidence for gene-smoking interaction

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It is recognized that the risk of bronchial asthma (BA) is conveyed by a complex interaction of genetic and environmental factors. The focus of this study was to investigate whether genetic variation of antioxidant defense enzymes (ADE) genes plays a role in the development of BA depending on the exposure to tobacco smoking. A total 429 DNA samples (214 asthmatics and 215 controls) from adult Russian population (Central Russia) were genotyped for polymorphisms of ADE genes: catalase (-262C>T, *CAT*), superoxide dismutase (Ala16Val, *SOD2*) and glutamate cysteine ligase (-588C>T, *GCLM*) genes through PCR-RFLP assays. It has been found that a genotype -588CT was associated with increased risk of nonallergic asthma (odds ratio 2.03 95% CI 1.05-3.90). No associations with BA were found for either the *CAT* or *SOD2* polymorphisms. Association analysis stratified by smoking status has revealed that a genotype -588CT of the *GCLM* gene was significantly associated with increased risk of nonallergic BA only in smokers (odds ratio 3.60 95%CI 1.42-9.10 p=0.01). The finding provides an additional evidence for the trigger role of tobacco smoking in the penetration of susceptibility to asthma determined by genes encoding antioxidant defense enzymes. The study was supported by the Federal Targeted Programme “Scientific and Scientific-Pedagogical Personnel of the Innovative Russia in 2009-2013”.

P09.044
Novel variants of beta-subunits of sodium channel genes in Brugada Syndrome

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Brugada syndrome (BrS) is a genetic disorder characterized by lethal ventricular fibrillation and right precordial ST segment elevation on ECG. Loss-of-function mutations in SCN5A cause 15-20% of BrS cases. We aimed to investigate whether mutations in the four beta-subunits of sodium channel genes (SCN1B to SCN4B) are associated with abnormal cardiac excitation in BrS.

Direct Sanger sequencing was performed in 16 clinically diagnosed BrS patients, 11 asymptomatic individuals with positive family history for BrS or sudden cardiac death (SCD) and one asymptomatic individual with a positive ajmaline test, none of whom had SCN5A mutations.

Genetic analysis revealed a novel intronic variant and 3 SNPs in SCN1B, two intronic SNPs and one 3’UTR SNP in SCN2B, a novel silent exonic variant and four 5’UTR, one silent exonic and two intronic SNPs in SCN3B, and a novel 5’UTR variant and 2 exonic silent SNPs in SCN4B.

In silico splicing analysis using Alamut (Interactive Biosoftware) predicted that the novel intronic variant in SCN1B and the exonic variant in SCN3B have no impact on splicing. The novel variant in the minimal promoter of SCN4B was detected in an asymptomatic young individual with a family history of SCD and a father clinically diagnosed as BrS. Detection of the variant within the family and control population is ongoing.

In conclusion, these novel variants and polymorphisms in the beta-subunits of sodium channel genes may have a direct or modifier effect on developing BrS, but these results need to be validated by further large scale and functional studies.

P09.045
Testing BST-2/Tetherin gene as candidate host factor involved on HIV-1 susceptibility and progression rate to AIDS

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BST-2/Tetherin/CD317/HM1.24 inhibits HIV-1 release from infected cells by retaining virions on cell surface. HIV-1 Vpu protein counteracts *BST-2/Tetherin* action by sequestering and promoting its degradation. We have tested *BST-2/Tetherin* variants rs3217318 (promoter 20bp-indel) and rs10415893 (3’UTR tagSNP) for association with HIV-1 infection and disease progression. Genotypes were obtained for a cohort of 353 subjects exposed to HIV-1 infection by injection drug use; 185 infected (HIV+) and 171 non-infected (ENI). In addition a cohort of 188 healthy subjects were used as controls. Disease progression of the 185 HIV+ subjects was recorded from the first HIV-1 positive test to December 1999. A first drop on CD4 cell-count <200/!L was considered as outcome for progression. From HIV+ subjects, 75 were progressors and 110 non-progressors. No differences were observed in genotype and allele distribution between HIV+, ENI and controls; nor for rs3217318 neither for rs10415893 (Table-1). However, rs10415893 allele distribution in non-progressors showed statistically significant differences when compared with progressors (P=0.045) and controls (P=0.008). In addition, prevalence of rs10415893-GG homozygotes was higher in non-progressors when compared with both, progressors (P=0.036; OR=0.47; 95%CI:0.2-1) and controls (P=0.005; OR=0.45; 95%CI:0.25-0.79). Finally, mean time to progression for rs10415893-GG was higher than for rs10415893-(GA+AA) subjects (150 *vs.* 120 months; Log-Rank test P=0.031; unadjusted Hazard-Ratio=0.6; 95%CI:0.36-0.96, P=0.033). Our results do not support a role of BST-2/Tetherin in HIV-1 susceptibility but suggest its involvement in progression.

Table 1.- Genotype and allele distribution of *BST-2/Tetherin* polymorphic variants in cohorts

			Prevalence in cohorts (n,%)				
					HIV-1 positive		
Variants ($)	Location		Controls	All	Progressors (*)	Non-Progressors	ENI
rs3217318	promoter	Genotype					
		WtWt	129 (69)	134 (73.6)	51 (68)	83 (77.6)	117 (68.4)
		WtD20	52 (27.8)	42 (23.1)	22 (29.3)	20 (18.7)	49 (28.7)
		D20D20	6 (3.2)	6 (3.2)	2 (2.7)	4 (3.7)	5 (2.9)
		Allele					
		Wt	310 (82.8)	310 (85.2)	124 (82.6)	186 (86.9)	283 (82.7)
		D20	64 (17.2)	54 (14.8)	26 (17.4)	28 (13.1)	59 (17.3)
rs10415893	3''UTR	Genotype					
		GG	128 (68.1)	143 (77.3)	52 (69.3)	91 (82.7)	128 (75.3)
		GA	55 (29.3)	38 (20.5)	21 (28)	17 (15.5)	38 (22.4)
		AA	5 (2.7)	4 (2.2)	2 (2.7)	2 (1.8)	4 (2.4)
		Allele					
		G	311 (82.7)	324 (87.5)	125 (83.3)	199 (90.4)	294 (86.5)
		A	65 (17.3)	46 (12.5)	25 (16.7)	21 (9.6)	46 (13.5)
($) Strong linkage disequilibrium between markers in all cohorts							
(*) Progresors were those falling CD4 cell count <200/μL							
ENI, HIV-1 exposed uninfected subjects							

P09.046
Is CADPS2 involved in autism and intellectual disability?

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We describe a family where brother and sister show mild intellectual disability, behavior abnormalities and epilepsy, with normal brain imaging and no major dysmorphic features. Karyotype is normal, as well FMR1 analysis in the male patient. Their mother, who died of cancer at 48 years of age, and a maternal aunt are both reported to have behavioral abnormalities, mild intellectual disability, but no seizures. Two maternal uncles are healthy.

In both patients, array-CGH analysis revealed a 250 kb deletion of chromosome 7q31.32 encompassing one single gene, CADPS2. The father does not carry the deletion, indicating likely maternal inheritance. The deletion was excluded in one of the maternal uncles.

CADPS2 is a calcium binding protein that regulates the exocytosis of synaptic and dense-core vesicles in neurons and neuroendocrine cells. CADPS2 is a candidate gene for autistic disorders, since it maps in the autism susceptibility region AUTS1 on chromosome 7q31.32, which alsocorresponds to a candidate region for mood disorders. Knock-out mice show an autistic-like phenotype and some ASD (Autism Spectrum Disorders) patients show a possible defect in CADPS2 splicing. We performed, therefore, a mutation screening in 46 individuals with ASD and in 34 patients with intellectual disability who did not carry any genomic rearrangement as far as array-CGH analysis could detect. Two novel variants were identified in two unrelated ASD individuals, in exon 6 and exon 13 respectively. None of these changes was detected in 140 healthy individuals and functional analysis of these variants is currently ongoing. Detailed results will be presented.

P09.047
Screening of TCF2 and PAX2 genes for mutations associated with congenital anomalies of the kidney and urinary tract in Bulgarian patients

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Congenital anomalies of the kidney and urinary tract (CAKUT) affect 1 in 500 children worldwide and are a leading cause of childhood end-stage renal disease. The two most common CAKUT diagnoses are the renal dysplasia and hypoplasia. The genetic background of these anomalies is still largely unknown. Several genes involved in urinary tract development have been implicated - HNF1b, PAX2, SIX2, etc. The molecular defects identified so far do not account for all the cases and cannot explain the great variability of phenotypes observed, which warrants further investigations.

In the present study we focused on two genes most commonly affected in CAKUT - TCF2 (HNF1b) and PAX2, which code for transcription factors with essential role for renal development. Defects in TCF2, most commonly large deletions, are shown to cause renal cysts, kidney dysplasia and sometimes renal agenesis, often in combination with diabetes mellitus. Point mutations in PAX2 cause renal hypoplasia and eye pathologies.

We report here on the introduction of TCF2 and PAX2 mutation screening for CAKUT patients in Bulgaria. Altogether 15 families were involved in the present study. Each of the affected individuals was tested for the presence of deletions or point mutations by means of MLPA analysis and sequencing of the coding sequences of TCF2 and PAX2. The identification of mutations in each of the affected families will facilitate the genetic counseling. Further screening for mutations in genes essential for genitourinary system development will be performed in those cases where no defects were found in TCF2 and PAX2.

P09.048
Lack of the association between T1128C variant in the neuropeptide Y gene and cannabis users

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Objectives of the Study: Substance addiction is complex and multifactorial disease that develops based on environmental and genetic factors. Cannabis that is the most frequently used substance. Several studies suggest that a possible involvement of neuropeptide Y (NPY) system in the physiological effects of abused substances including alcohol and cocaine. The aim of this study was to examine whether T1128C functional variant of the NPY gene are associated with cannabis dependence.

Methods: In our study, 139 cannabis users and 100 healthy controls were tested for T1128C variant of NPY gene. Genotyping were performed by PCR-RFLP and the allele frequency of healthy people in Turkey was compared with the other country by two proportions z test.

Results: The genotype distribution of T1128C of NPY gene was not significantly different from that expected according to Hardy-Weinberg equilibrium in any group. There was no significant difference in the genotype distribution and allele frequency of T1128C variant between cannabis users and controls ($p>0.05$).

When the 1128C rare allele frequency of healthy people is compared with other country; it has been determined that it is coherent with America, China, Germany, Brazil, Sweden, Netherlands and Finland ($p>0.05$). On the contrary, the results obtained were different than the Japan ($p=0.022$).

Conclusion: This study is the first to search NPY gene in cannabinoid users. NPY gene T1128C variant did not reveal any relationship with the cannabis addiction. It may, therefore, be useful for considering to study NPY receptor genes for elucidating the genomic basis of addiction.

P09.049
Association study of CARD-8 (p.C10X) and NLRP3 (p.Q705K) variants with Rheumatoid Arthritis in French population

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Objective: NALP3, ASC, and TUCAN are components of the NALP3 inflammasome, a crucial molecular platform that regulates activation of caspase-1 and processing of interleukin (IL)-1 beta and IL-18. We undertook this study to investigate the association of caspase activating and recruitment domain 8 (CARD-8) and NLR family, pyrin domain containing 3 (NLRP3) polymorphisms with rheumatoid arthritis (RA) in French population.

Methods: CARD8 (c.30T>A, rs2043211) and NLRP3 (c.2113C>A, rs35829419) single nucleotide polymorphisms (SNPs) were genotyped in 100 French RA trio families, with the 4 grandparents of French Caucasian origin, using TaqMan 5' allelic discrimination assay on an ABI 7500 real time PCR machine (assay: C__11708080_1_ and C__25648615_10; respectively). The genetic analyses for association and linkage were performed using the comparison of allelic frequencies (AFBAC), the genotype relative risk (GRR), and the transmission disequilibrium test (TDT).

Results: Our results did not show any significant association either with CARD-8 [p = 0.894, odds ratio (OR) 1 (95% CI 0.41-2.43)] or NLRP3 [p = 0.879, odds ratio (OR) 0.87 (95% CI 0.3-2.48)] genes and RA. Moreover, stratifying patients according to the presence of rheumatoid factor (RF), anti-cyclic peptides antibodies (ACPA) and erosion did not reveal any significant association neither with CARD-8 nor with NLRP3 (p>0.05).

Conclusion: Variations in the innate immunity genes CARD-8 (p.C10X) and NLRP3 (p.Q705K) have no effect on RA susceptibility in the French population.

P09.050
Impact of selected SNPs from recent GWAS on plasma lipid concentrations in a high cardiovascular risk Mediterranean population. Interaction with environmental factors

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A recent meta-analysis of genome-wide association studies (GWAS) has revealed new statistically significant association between dozens of SNPs and plasma lipid concentrations including thousands of individuals from diverse populations. However, despite the great interest of these newly discovered significant associations, one of the potential limitations may be the low impact of these SNPs on a single population in terms of clinical relevance and not only in statistical significance. Therefore our aim was to analyze the clinical relevance (measured in terms of consistency and magnitude) of the association between some of these GWAS-SNPs reported as significantly associated with lipid traits (total cholesterol, LDL-C, HDL-C or/and triglycerides) in a high cardiovascular risk Mediterranean population. We randomly selected 20 SNPs from the recent meta-analysis (Nature, 2010) and genotyped these SNPs in 1050 high cardiovascular risk subjects (aged 67+/-7 years) participating in the PREDIMED-Valencia Study. We analyzed their associations with lipid traits as well as some gene-environment interactions. All the randomly selected SNPs (rs629301, rs1689800, rs2642442, rs514230, rs7570971, rs645040, rs13107325, rs1800562, rs3177928, rs605066, rs2072183, rs4731702, rs9987289, rs2293889, rs7941030, rs7134375, rs8017377, rs2412710, rs11649653 and rs2925979) were in Hardy-Weinberg equilibrium. The replication rate in terms of statistical significance (P<0.05) of the association of each SNP with the main trait, was low. However, we were able to detect some consistent associations (rs629301 in *SORT*1 and LDL-C; rs2642442 in *MOSC1* and total cholesterol, etc). In addition we found various statistically significant interactions with tobacco, alcohol and adherence to the Mediterranean diet that may contribute to explain the heterogeneity.

P09.051
PPARGC1B is a Genetic Determinant of the Cardiovascular Risk Factor, Thromboxane A_2 - an Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) Sub-study

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Introduction: Thromboxane A_2 (TxA_2), activates platelets and causes vasoconstriction. Raised urinary excretion of 11-dehydro-thromboxane B_2 (TxM), an index of TxA2 formation, is an independent predictor of atherothrombotic events. The heritability of this trait is not known. This is the first study to investigate the genetic determinants of thromboxane levels.

Materials and Methods: 544 participants in the Hypertension Associated Cardiovascular Disease Sub-study of ASCOT gave urine samples at two separate time-points. TxM was measured using liquid chromatography/ tandem mass spectrometry, expressed as pg/mg creatinine to correct for urine concentration. All subjects were genotyped on the CVD50K chip, a cardiovascular-specific chip with >50,000 SNPs. Usual quality control measures were applied. Linear regression analysis was performed, assuming an additive genetic model, adjusting for the covariates; age, sex, smoking habit, diabetes, systolic blood pressure, BMI, HDL, LDL, aspirin and anti-hypertensive regimen.

Results & Discussion: TxM levels were significantly lower in patients taking aspirin versus aspirin naïve patients (median [interquartile range] 324 [211,404] *vs* 716 [460,913] pg/mg, p<0.0001). 22 SNPs in the PPARGC1B gene were significantly associated with higher TxM (beta-values range; 123-223 pg/mg, p-values range; 0.001-0.009 after permutation to correct for multiple testing). PPARGC1B encodes peroxisome proliferator-activated receptor gamma co-activator 1β, a transcriptional co-activator previously reported to be associated with type 2 diabetes and obesity. These findings provide novel insight into the molecular biology of TxA2 biosynthesis, and its role in the causation of heart attacks and strokes.

P09.052
In-depth assessment of cardiovascular parameters in three canine breeds as a preliminary step to unravel genetic determinants of physiological blood pressure variation.

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Chronic values of arterial blood pressure (ABP) outside normal ranges represent a risk factor associated with cardiovascular disorders, a main cause of death in Europe. Deciphering and reversing complex morbidity mechanisms will require identifying the genetic polymorphisms that eventually contribute to systemic arterial hypertension. The genetic basis of arterial stiffness in humans, for example, has been extensively investigated through genome-wide association studies (GWAS), and this has helped identify groups of relevant genes unrelated to previously suspected mechanisms.

In parallel, comparative pathophysiology between humans and animal models has been proved relevant. In particular, the dog is unique to map loci underlying complex traits. To improve the current knowledge on the functional network underlying variations in ABP, we decided to focus on the molecular basis of physiological variations of this variable in healthy dogs. Eighty-nine dogs (age: 3.4±1.2 years, range: 1.4-5.7) were prospectively recruited. Three breeds were represented (Labrador, n=30; Doberman, n=31; Belgian Shepherd, n=28). Dogs were considered healthy on the basis of a complete clinical, biochemical and echocardiographic and Doppler examination. Using high-definition oscillometry, we showed that systolic, diastolic and mean ABP in Dobermans was significantly higher than in Belgian Shepherds and Labradors (P<0.0001). ABP was also significantly higher in Belgian Shepherds than in Labradors (P<0.0001). DNA from these dogs was

extracted and GWAS analyses to map blood pressure loci will be conducted as part of the European LUPA project (www.eurolupa.org). In addition, heart rate and shortening fraction were similar between breeds, providing clues to further select specific candidate genes.

P09.053
The Human Caveolin 1 Gene Upstream Purine Complex and Neurodegenerative disorders - A Common Signature

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The caveolin 1 gene (*CAV1*) is over-expressed in experimental animal models of multiple sclerosis (MS). Increased expression of this gene has also been reported in the Alzheimer's disease (AD) brain. Loss of this gene, on the other hand, has recently been reported to be associated with neruodegeneration. We have recently reported a polymorphic purine complex at the 1.5kb upstream sequence of the gene with GGAA and GAAA motifs. In this study we compared the homozygote haplotype compartment across the region in groups of late-onset AD (n=240), MS (n=246), and control (n=610) subjects. Nineteen haplotypes were found to be homozygous in the patients, and not in the control pool ($p<0.000001$). The homozygote haplotypes ranged from 86-bp to 142-bp in the patients, whereas this range was between 106-bp to 122-bp in the controls. Six overlapping homozygote haplotypes were observed between AD and MS patients, strengthening the role of this region as a common etiological factor in the pathophysiology of neurodegenerative disorders, possibly through inflammatory mechanisms. The *CAV1* purine complex GGAA and GAAA motifs contain binding sites for numerous inflammatory transcription factors including the Ets, STAT, and IRF family members. Further work on the functionality of this region will shed light on the downstream events to the linked haplotypes.

P09.054
Variants of the CD36 gene and metabolic syndrome in Iranians

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Introduction: The CD36 gene encodes for a membrane receptor that facilitates fatty-acid uptake and utilization. Genetic variants of CD36 gene have been associated with the metabolic syndrome (MetS). We aimed to evaluate the association between the rs10499859 and rs13246513 polymorphisms and MetS components.

Methods: For this case-control study, 140 MetS and 187 normal subjects were randomly selected from Tehran lipid and Glucose Study participants. Biochemical and anthropometrical variables were measured. Genotyping was performed by PCR-RFLP.

Results: Participants' mean age was 69 years and 56.3% were male. Case and control groups were not different in allele and genotype frequencies for these SNPs. However, the presence of T allele in rs13246513 C>T was significantly associated with increased levels of HDL-C (p 0.016) using a dominant model; this association was no longer statistically significant after adjustment for sex and age (p 0.751). The association of rs10499859 with diastolic blood pressure was significant (p 0.021), but disappeared after adjustment for sex and age, using recessive model (p 0.133). Association between this SNP and BMI was significant after adjustment for MetS under the dominant model (p 0.009, β^2=0.68). With regard to other MetS parameters, no significant difference was observed between genotype groups.

Conclusion: We found no difference in allele frequencies of rs10499859 and rs13246513 polymorphisms between case and control groups, indicating possibly no association between these polymorphisms and MetS. However, based on our results, these polymorphisms do affect HDL-C level and BMI, although the effect may be slight and restricted specifically to an environment-genotype.

P09.055
Copy number analysis of functional candidate genes on celiac disease linkage regions

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INTRODUCTION: Celiac disease (CD) is a complex, immune-mediated intolerance to dietary gluten that develops in genetically susceptible individuals. Different strategies including candidate gene association, linkage and GWAS have been employed to identify common susceptibility variation. CNV affects approximately 12% of the genome and gene dosage variation within a CNV could influence expression and disease susceptibility, but systematic studies in CD are lacking.

METHODS: The chromosomal location of significantly altered genes from a previous expression microarray experiment (9 active CD patients *vs.* 9 patients on gluten free diet) was crossed with the CD linkage regions described in the literature. Genes located within copy number variable regions present in >5% of individuals (Database of Genomic Variants, projects.tcag.ca/variation/) were selected for CNV analysis by gene-specific real-time PCR in 450 CD patients and 443 non-CD controls and assigned a predicted copy number for each sample using the maximum likelyhood approach.

RESULTS AND COMMENTS: Our candidate gene identification strategy allowed us to select 6 genes for CNV analysis: *DAZAP1*, *MBOAT7*, *PEX26*, *PRELID1*, *SYNPO* and *ZSCAN18*. To date, our results indicate that *PEX26* copy number does not vary in our population. Preliminary data suggest that ≥3 copy diplotype of *PRELID1* (overexpressed in active CD mucosa) is overrepresented in the patient group, indicating a possible association of *PRELID1* high copy number with CD risk. We are currently analyzing the rest of our candidate genes.

P09.056
Enrichment of rare coding variants in genes comprising the 39 loci associated to celiac disease

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Celiac disease (CD) is a complex disease triggered by dietary gluten, resulting in an inflammatory response in the small intestine. It is known that human leukocyte antigen (HLA) DQ2/DQ8explains approximately 30% of CD heritability. Genome wide association studies (GWAS) identified 39 non-HLA CD loci explaining an additional 5% of the missing heritability. These 39 non-HLA loci comprise113 genes that could potentially also contain rare variants contributing to CD. GWAS arrays preclude the identification of these rare variants. In this study, high-throughput exome sequencing was used to investigate significant enrichment of novel and rare coding variants in CD cases versus controls.

Genomic DNA of15 unrelated individuals with CD was enriched for the ~30Mb of coding DNA with NimbleGen 2.1M Human Exome Arrays and sequenced using Illumina technology. Sequence variants (SV) with a quality score above 20 were annotated with our custom pipeline. On average, 91% of the exome was covered at least ten-fold and on average18.468genetic variants were identified per individual. To find rare and novel variants in the regions of interest our analysis was restricted to the 113 genes from the 39 CD loci. After removing common SV (>5%) based on HapMap and the 1000 Genome project and all SV present within non-coding regions, we are left with 20 potential variants. To perform statistical analysis in a case-control design we are currently analyzing some 100exomes from Dutch controls with the same prioritization steps.

P09.057

Upregulation of KIR3DL1 gene expression in intestinal mucosa in active celiac disease

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INTRODUCTION: Celiac disease (CD) is a complex, immune-mediated intolerance to gluten that develops in genetically susceptible individuals and involves both innate and adaptive immune responses. Killer cell immunoglobulin-like receptors (KIRs) modulate NK and T-cell function through HLA class I interaction and have been implicated in CD.

METHODS: Qualitative expression of 14 KIR genes and 2 KIR pseudogenes was determined by SSP-PCR in biopsy pairs from 22 CD patients (taken at diagnosis and after two years on gluten free diet-GFD). Quantitative expression analysis of *KIR2DL4*, *KIR3DL1*, *KIR3DL3* and *KLRC2* (a marker of an NK-reprogrammed T-cell subpopulation augmented in CD) was performed in 35 additional CD biopsy pairs and 14 non-CD control biopsies by gene specific RT-PCR. Qualitative expression differences between groups were analyzed in 2x2 contingency tables, while expression levels were compared by paired (diagnosis/GFD) and unpaired (CD/controls) T-tests.

RESULTS: No particular KIR expression profile was observed for the different CD stages. *KIR3DL1* was more frequently expressed in active CD compared to GFD (p=0.0312) and non-CD controls (p=0.0008) with slightly increased levels in active disease (p=0.0505). *KLRC2* was overexpressed in active (p=0.0037) and GFD (p=0.0469) patients compared to non-CD controls and coexpressed with *KIR3DL1* (r^2=0.2917; p=0.001574).

DISCUSSION: Results suggest an alternative activating role for *KIR3DL1* in CD mucosa, as seen in other autoimmune diseases such as type 1 diabetes. *KIR3DL1* expression in CD could be explained in part by the increase in NK-like T cell subpopulations, but other mechanisms need to be involved.

P09.058

Contrasting the genetic background of type 1 diabetes and celiac disease autoimmunity

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Type 1 diabetes (T1D) and celiac disease (CD) cluster in families and co-occur in the same patients. The same genetic regions and even the same SNPs have been associated with both diseases. Our aim was to test the genetic differences between individuals developing both diseases and those having only T1D or only CD.

Analysis1: We genotyped 32 established CD and/or T1D associated SNPs in 1115 non-Hispanic white American (NHWA) T1D patients and 260 NHWA T1D patients with also CD-autoimmunity (CDA&T1D) defined as persistent tissue-transglutaminase positivity on 2 consecutive visits. CD private risk variants in the *LPP* and *CD80* loci are associated with developing CDA&T1D when compared to T1D. Interestingly, IL18RAP*A - which is a risk allele for CD and a protective allele for T1D - was more frequently observed in the CDA&T1D group (Table).

Analysis2: Genotypes of 29 SNPs were available for 803 Dutch CD patients biopsy confirmed and showed the same allele frequencies in NHWA and Dutch controls. We compared the NHWA CDA&T1D to the Dutch CD patients. CDA&T1D individuals carried more frequently the T1D *PTPN22* risk variant and less the CD *ETS1* and *IL12A* risk variants when compared to CD individuals (Table).

This pilot study shows that to develop both diseases, individuals accumulate private T1D and CD risk variants. To improve our study, we are currently increasing sample sizes and numbers of SNPs.

			Non-Hispanic white American CDA&T1D vs T1D only					
Loci	SNP	OR_GWAS	A1#	Freq(T1D & CD) N=260	Freq(T1D only) N=1115	A2	P	OR
HLA-DQ2.5	rs2187668_CD and T1D	6.23	A	0.44	0.27	G	7.1E-14	2.10 [1.73-2.56]
LPP	rs1464510_CD	1.29	A	0.51	0.44	C	3.5E-03	1.33 [1.10-1.61]
CD80	rs11712165_CD	1.13	C	0.43	0.38	A	2.8E-02	1.24 [1.02-1.51]
IL18RAP	rs917997_CD and T1D	1.19	A*	0.27	0.22	G	7.5E-03	1.34 [1.08-1.67]
CTLA4	rs3087243_T1D	0.82	A	0.32	0.41	G	6.6E-04	0.70 [0.58-0.86]
			Non-Hispanic White American CDA&T1D vs Dutch CD only					
Loci	SNP_Associated disease	OR_GWAS	A1	Freq(T1D & CD) N=260	Freq(CD only) N=803	A2	P	OR
HLA-DQ2.5	rs2187668_CD and T1D	6.23	A	0.44	0.55	G	3.8E-06	0.63 [0.51-0.76]
ETS1	rs11221332_CD	1.21	A	0.21	0.25	G	2.6E-02	0.76 [0.60-0.97]
IL12A, SCHIP1	rs17810546_CD	1.36	G	0.11	0.17	A	1.7E-03	0.62 [0.46-0.84]
IL12A	rs9811792_CD	1.21	G	0.45	0.50	A	4.2E-02	0.81 [0.67-0.99]
PTPN22	rs2476601_T1D	2.05	A	0.18	0.10	G	1.7E-06	1.96 [1.49-2.60]

Freq = Frequency; N = number; A1# = same alleles reported by the GWAS; GWAS = genome-wide association studies

P09.059**

Searching for causative variants in a large family with celiac disease

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Celiac disease (CD), caused by an immune response to gluten, is a common food intolerance. CD is strongly associated to human leukocyte antigen (HLA) DQ2/DQ8. A genome-wide association study identified 39 loci which together with HLA explain 35% of CD heritability. To identify additional CD genes we performed whole-genome linkage in a four-generation Dutch family with 17 CD patients. We identified linkage to a 6Mb region on 9p21-13 (dominant model) and to a 14Mb region on 6q25 (identity by descent sharing). We hypothesize that these regions contain high risk mutations, playing a causal role in disease development in this family.

To identify potentially damaging mutations we sequenced the exomes of two affected family members, separated by four meiotic steps. Genomic DNA was enriched for coding regions using an Agilent SureSelect target kit and sequenced on a Genome Analyzer II (Illumina). On average 70% of the exomes were covered at least ten-fold and we observed ~15,000 sequence variants per individual. To reduce the number of variants we a) discarded common variants (MAF>5%) based on HapMap and the 1000Genomes Project, b) removed all non-coding variants, c) analyzed PolyPhen prediction and/or d) select on gene function. This left us with four variants (one on 9p21-13 and three on 6q25), that were validated by Sanger sequencing. The two variants that also segregated in the family are currently genotyped in a

large case-control cohort to test the association to celiac disease. Both variants map to genes involved in the transport of molecules.

P09.060
Association of the IL33 gene region with childhood allergic asthma

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Two recent indipendent large-scale GWAS of asthma reported the association of several markers comprising some on chromosome 9. In this region, flanking IL33 gene, we have previously found linkage of marker D9S286 with elevated IgE and SPT in Italian asthmatic families.

Here we report a family transmission study with allergic asthma in 137 trios from North-Eastern Italy ascertained through an allergic asthmatic child. Association analyses was conducted by the transmission disequilibrium test (TDT).

The phenotypes clinical asthma, total serum IgE levels, bronchial hyper-responsiveness to methacoline (BHR+), skin prick test positivity to common aeroallergens (SPT+), to house dust mites (SPT-HDM), and to graminae (SPT-GMN) were investigated.

The SNPs rs1342326 and rs928413 analysed, were in Hardy-Weinberg equilibrium and in LD with each other (D'=0.95; R^2=0.59). The observed minor allele frequency was 23% (minor allele:G, major allele:T) and 31% (minor allele:G, major allele:A) for rs1342326 and rs928413.

TDT analysis showed a preferential transmission of the rs928413-G allele to individual with SPT (T:65, NT:43, p=0.034) and in particular with SPT-GMN (T:54, NT:33, p=0.024). No other association was found.

Haplotype analyses showed that rs1342326-rs928413/T-G was preferentially transmitted to children affected by asthma (p=0.018), BHR+ (p=0.006), IgE (p=0.022), or SPT (p=0.015) and in particular by SPT-GMN (p=0.006).

These results extend the association of 9p24 with allergic asthma traits indicating the possible role of IL33 as an "alarmin" signal to immune system.

P09.061
Twelve common genetic variants explain a substantial part of the variation in BMI of obese children

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Results of twin and family studies suggest that up to 80% of the variation in body mass index (BMI) in children can be explained by genetic factors. A recent study (*Diabetes* 59:2980-2988) investigated 12 single nucleotide polymorphisms (SNPs) that are robustly associated with adult BMI in a large pediatric population. Direction-consistent associations with BMI were found for all of these variants in children. However, the risk factors explained a disappointing 1% of the population variation in BMI when taken together. We hypothesized that these 12 SNPs also explain variation in BMI in obese children. We studied 64 patients from our pediatric obesity clinic to test this hypothesis (39% boys; mean age=13.2; age range: 6 to 19; all children of North-West European descent). BMI-standard deviation scores (BMI-SDS) were used as a measure of body fat percentage of the children. BMI-SDS indicates how many standard deviations the child is away from the mean of a reference population of the same age and sex. Mean BMI-SDS of the study group was +2.8 (range: +1.9 to +3.8).The SNPs were genotyped using pre-designed TaqMan assays. We calculated a genetic predisposition score for each subject, i.e. the number of BMI-increasing alleles (theoretical range: 0 to 24). The genetic predisposition score significantly predicted BMI-SDS in this group of obese children (*p*=.02). The score explained 8.4% of the variation in BMI-SDS in the group. The largest effect size was found for the fat mass and obesity associated gene (*FTO*, beta=+0.2). Future plans include increasing the sample size.

P09.062
CHRNA4 exon 5 variant affects nicotinic receptor sensitivity and is associated with schizophrenia-related quantitative traits and drug treatment-response

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Background: Association of CHRNA4 exon5 SNP rs1044396 with cognitive performance, associated brain function and nicotine addiction has been reported. At present, it is unclear whether rs1044396 is functional. Since patients with schizophrenia are mostly strong smokers showing cognitive deficits and abnormal brain function, CHRNA4 also might be a potential candidate gene. Methods: cDNAs (incl. rs1044396) carrying two complementary haplotypes (hap1, hap2) were injected into Xenopus Oocytes. The alpha4beta2 receptor channel was studied with voltage clamp applying acetylcholine (Ach) concentrations in increasing order. Case-control and quantitative trait association study (N = 1.253 schizophrenia cases and N = 2.880). Pharmacogenetic study in two independent sample (total: N = 220 schizophrenia patients) testing for association with treatment-response. Population-based association study of cognitive performance and brain function (functional neuroimaging) during a choice reaction task in N = 1.794 smoking and never-smoking healthy subjects. Structural magnetic resonance imaging (MRI) in N = 122 healthy subjects testing for association with cortical thickness. Results: 6% stronger current amplitudes in alpha4beta2 receptor channels in response to low Ach doses depending on haplotype. The opposite effect was seen with higher Ach doses. Schizophrenia, clinical symptoms and clinical response to drug treatment were significantly associated with genotype. Significant genotype association with verbal memory interacting with age and sex as well as brain function, task-related reaction time and cortical thickness of the medial prefrontal lobe. Discussion: Experimental evidence is provided that rs1044396 affects nicotinic alpha4beta2 receptor sensitivity. The association findings imply a role in schizophrenia pathophysiology and drug-treatment response.

P09.063
FN1 and *TIMP2* polymorphisms are associated with nonsyndromic cleft lip with or without cleft palate

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Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is a common human birth defect with complex genetic etiology. The aim of this study was to investigate the contribution of 12 candidate genes (*FGF1*, *FGF2*, *FGFR1*, *FN1*, *MMP2*, *MMP3*, *MMP9*, *MMP25*, *TIMP2*, *TIMP3*, *WNT3*, and *WNT9B*) in predisposition to orofacial clefting. 209 tagSNPs were studied in 192 NSCL/P patients and 424 controls from Estonian and Lithuanian population. The genetic relatedness of Estonians and Lithuanians, sharing the same geographic origin, has been recently confirmed using the principal component analysis. In case-control comparisons, the minor allele of *FN1* SNP rs4968282 was associated with reduced risk of NSCL/P (OR=0.653; 95% CI 0.511-0.836; $P=6.73\times10^{-4}$), whereas the minor allele of *TIMP2* rs7502916 was associated with increased risk of NSCL/P (OR=1.465; 95% CI 1.150-1.867; P=0.0019). The minor allele of *MMP9* SNP rs6073991 was associated with reduced risk of NSCL/P (OR=0.648; 95% CI 0.480-0.874; P=0.0043). Multiple yin-yang haplotypes in *FN1* and *TIMP2* were associated with NSCL/P. The strongest associations were found for *TIMP2* haplotype CAA ($P=8.06\times10^{-4}$) and *FN1* haplotype GAA ($P=9.08\times10^{-4}$). Fibronectin is involved in cell adhesion and migration processes during embryogenesis, and the present study is the first to demonstrate an association between common SNPs and haplotypes in *FN1* and NSCL/P. Our findings provide evidence that variation in *FN1* and *TIMP2* may contribute susceptibility to NSCL/P in North-Eastern European populations.

P09.064
Genetic evidence supporting a role for MMP and TIMP genes in Cleft Lip/Palate

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The complex etiology of cleft lip/palate (CL/P) is acknowledged by the number of genes and signaling pathways implicated during craniofacial development. Matrix metalloproteinases (MMPs) and their inhibitors TIMPs are responsible for degradation of the extracellular matrix and tissue remodeling during including embryonic development and may be implicated in CL/P. We have previously shown that a polymorphism in MMP3 is associated to CL/P. We genotyped 45 polymorphisms spanning selected MMP and TIMP genes in 907 unrelated Caucasian individuals. Statistical analyses were performed using PLINK software. Genotype and allele frequencies of polymorphisms were compared between cases and controls under Bonferroni correction (α=0.0016). Odds-ratios and 95% confidence intervals were also calculated. MMP3 rs522616 showed statistically significant association with all clefts (P=0.000006), cleft lip with cleft palate (P=0.0003) and with cleft palate (P=0.0002). TIMP2 rs8179096 showed association with all clefts (P=0.0007) and with cleft palate (P=0.001). Logistic regression analyses reinforced the individual associations. In silico analyses for both MMP3 and TIMP2 variants revealed several transcription factor binding sites relevant for craniofacial development. We also report for the first time the expression pattern of MMPs not yet described during murine palatogenesis. In summary, we provide additional evidence that MMP3 and also TIMP2 contribute to CL/P. Additional studies are under way to elucidate the involvement of these genes in the etiology of CL/P.

P09.065
Independent rare and common variants underly association signals for coeliac disease

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Coeliac disease is an autoimmune disease with 1% prevalence in the general population and a complex genetic background. Our recent genome wide association scan (GWAS) on 15,283 case-control samples established association to 27 loci, including well known coeliac risk factor in the HLA region. Currently we genotyped 24,269 case-control samples (UK, Netherlands, Italy, Spain, Poland, India) on the Immunochip; a custom Illumina iSelect platform (196,548 variants) designed with aim of using all 1000Genomes-pilot markers to fine-map 189 risk loci reported at genome wide significance for over ten immune mediated diseases.

In the meta-analysis across all populations we observed associations (p<5x10e-8) at 14 additional loci, making a total of 41 coeliac disease risk loci. Moreover, we noted an excess of intermediate range p-values at the remaining 148 autoimmune loci, confirming the large genetic overlap between immune-mediated diseases. Finally, to get better insight into the genetic architecture underlying associations for previously established coeliac disease loci, we performed a stepwise conditional logistic regression on all 26 GWAS regions (20-50x more variants per locus on Immunochip compared to GWAS chips). Nearly half of the loci (12 of 26) showed evidence for multiple independent signals, due to both common (e.g. 3 signals at IL12A) and rare genetic variants (MAF< 0.05, e.g. at PTPN2).

Identification of multiple independent SNPs at coeliac disease associated loci helps to better define the risk of each individual locus, understand the causal mechanism behind the associated variants and provides insights into the likely genetic architecture of other common complex diseases.

P09.066
Incidence of TACI mutation in common variable immunodeficiency

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Background: Common variable immunodeficiency disorders (CVID) are heterogeneous immunodeficiency syndromes. Heterozygoute mutations in TACI gene have previously been shown to cause recessive CVID. The aim of this study was to determine the incidence of TACI defect in CVID patients.

Method: 18 unrelated CVID patients were entered the study. PCR amplification was performed using unpublished primers, to amplify TACI.

Table1. Primers used in this study

Forward primer	Reverse primer
5'-AGCCCAAGCACTAATCAAATCTAA-3'	5'-GGCCCAACCCTCCTCACAC5-3'
5'-GCCGCCTGCCAGCTGCCCTCAC -3'	5'-GCCGCCTGCCAGCTGCCCTCAC -3'
5'-CACCCAGCAGTATCATGAATTTCT-3'	5'-AGGCCTCCCACGCTTTCTCAC -3'
5'-GGGGGATGTGGATTGCTTGAG-3'	5'- GGCGAGGACCCCAGTTTCATG-3'
5'-TGCCCACACCGTCACCCCTACC-3'	5'-TCCCCTCCTCTCCATCTCTCTC -3'

Result:

Table2. Patient's data and the result of TACI mutation screening

Patient number/ sex	Age(year)	TACI mutation	Affected TACI domain	IgA levels (mg/dl) (adults:71-308)	IgG levels (mg/dl) (adults:658-1837)	IgM levels (mg/dl) (adults:40-263)
1/male	48	R122W (reported)	Extra-cellular domain	7	755	15
2/male	28	38insA Frameshift(new)	Extra-cellular domain	238	216	233
3/male	17	38insA frameshift(new)	Extra-cellular domain	16	743	48

Conclusion: In previous studies, the proportion of CVID patients carrying at least one TACI mutation was estimated at 10 to 20%. In the present study, we identified 3 patients with at least one TACI allele mutated out of a small cohort of 18 antibody deficiency patients, yielding a more precise estimate of 16.6%. None of these mutations were present in 20 healthy subjects. This study suggests that additional genetic and environmental factors might have effective role in causing CVID. Analysis of a larger sample of patients will be needed to determine if the novel mutation associated with a particular phenotype or predisposition to the common features of CVID.

P09.067***
Increased genomic copy number of ß-defensins on 8p23.1 and its influence on psoriasis

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Previously, we reported association of an increased genomic copy number of a ß-defensin cluster on 8p23.1 with psoriasis (Hollox et al. Nat Genet 2008). In the meantime, our findings have neither been replicated nor rejected by independent studies, probably due to technical difficulties of differentiating copy number states of more than four copies.

In order to check the relevance of the association finding for psoriasis, we performed extensive methodological comparisons and an independent replication study. We observed satisfying correlation coefficients between three methods used: R^2 = 0.92 for MLPA and classical PRT (n=159), R^2 = 0.93 for classical and new PRT (n=68)). Furthermore, copy numbers obtained by a high resolution SNP array

(Affymetrix 6.0) were compared with PRT copy numbers in 600 individuals. No correlation was observed indicating that the array technique is not a suitable method to detect smaller copy number differences at loci with highly variable copy numbers.
We then re-evaluated findings in the German case control cohort as published in Hollox et al. (Nat Genet 2008) and confirmed the association with a p = 1.22E-05 (Mann-Whitney-test for unrounded copy number data). For the replication study we genotyped 1315 psoriasis patients and 593 control individuals. Statistical analyses of unrounded copy number data replicated the association with higher copy number in psoriasis (p = 3.46E-03). A combination of previous and replication datasets (obtained with purified DNAs only) resulted in a p-value of 9.94E-07. Overall, our study confirms association of psoriasis with increased copy number of ß-defensin cluster on 8p23.1.

P09.068
Copy number variation analysis by high resolution SNP array in cleft lip and palate

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High resolution SNP array technology has provided insights into a type of genomic variation known as copy number variation (CNV). CNVs have been detected in phenotypically normal individuals as well as associated with diseases and human malformations. Over the years it has been shown that the etiology of cleft lip and/or palate (CL/P) may be related to sequence mutations in specific genes, but also to copy number abnormalities such as deletions or duplications. The purpose of this study was to determine the role of CNVs in a group of CL/P patients (syndromic and nonsyndromic) composed by 23 proband-parents trio. To screen the CNVs, we used the Genome-Wide Human SNP Array 6.0 (Affymetrix). The analysis was performed using the Affymetrix Genotyping Console software and 20 in-house control samples was the reference set. By focusing on large events (>100 kbp), we observed an excess of 325 CNVs in patients (CNV average number per patient was 14). Of these, 126 were de novo CNVs. Duplications were more frequent than deletions. Approximately 150 CNVs involved known genes, including OMIM referenced genes. Among more interesting findings there are CNVs in regions known to be associated to CL/P phenotype such as a deletion (560kb) within the 1p36.33 microdeletion syndrome region, a deletion (300kb) within the 22q11.2 deletion syndrome critical region and a patient with syndromic cleft palate with 8p23.1 duplication (3.8Mb) and 15q25-q26 duplication (17Mb). Further experiments, by a second method to validate these CNVs found are currently being performed. **Financial support: FAPESP, CNPq.**

P09.069
Effects of ACE polymorphisms on severity of coronary artery diseases may be related with hyperlipidemia

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Introduction: Coronary artery disease (CAD) is a multifactorial disease which is influenced by genetic predisposition and environmental factors. Major risk factors of CAD are hypertension, diabetes mellitus, hyperlipidemia, smoking, family history and obesity. Mutations in ACE gene can also cause CAD. Thus, the aim of this study was to investigate the effects of major risk factors and ACE gene polymorphisms on CAD.
Methods: 205 consecutive patients with angiographically proven early onset CAD and also 140 control subjects with no significant coronary obstruction angiographically were recruited to the study. DNA was extracted from peripheral blood and ACE gene polymorphisms were investigated with PCR.
Results: The DD polymorphism was found statistically higher in patients than controls. Also ID polymorphism was found statistically high in patients, who have hyperlipidemia and smoking habits.
Conclusion: Many studies showed that the ID and DD polymorphism is strongly associated with the increased plasma or serum ACE levels. Thus the ID and DD polymorphism favors high ACE expression and activity, hence may predispose individuals to CAD and its complications. Although it has been suggested that hyperlipidemia is an independent risk factor for CAD, our findings showed a relationship between ID genotype and hyperlipidemia therefore the severity of CAD may be associated with hyperlipidemia. Also according to our results we consider that the combination of ID polymorphism with smoking habit may increase the development of CAD.

P09.070
Polymorphism +45T/G in ADIPOQ gene is not associated with ischemic heart disease in Central-European population

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Adiponectin harbours strong cardioprotective properties and the +45 T/G polymorphism in ADIPOQ locus has been reported to play an important role in diabetes development. The aim of the study was to investigate possible relationships between the SNP and coronary artery disease (CAD) in a large cohort of Central-European subjects with suspected CAD who underwent coronarography.
The 852 patients with suspected CAD referred for angiography were enrolled in the study, out of whom the 669 subjects presented with more than 50% reduction of coronary artery diameter and were classified as CADs (group 1) and 183 subjects were classified as the non-CADs (group 2). The healthy control subjects (n = 395) of the same age and gender distribution were recruited to establish normal population frequencies (group 3).
There were no significant differences in genotype distributions (p = 0.10) or in allele frequencies (p = 0.88) of +45T/G between the CADs and non-CADs (group 1 vs. group 2). However, there were significant differences in genotype distributions between subjects with coronarography (group 1+2) and without coronarography (group 3) (pg = 0.02, pa = 0.42). In multinomial regression modelling across the CADs, the +45 T/G polymorphism showed no association with ejection fraction (p = 0.87), number of affected vessels (p = 0.35) or number of stenotic lesions (p = 0.54)
Based on our observations, the +45T/G in ADIPOQ gene doesn't seem to be a major genetic determinant of CAD in the Central-European population, which contradicts findings on other CAD populations with different ethnicity.

P09.071
A genetic variant associated with the Cripto plasma levels in the Cilento isolated populations

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Cripto, the founder member of the EGF-CFC genes, has a key role for the Vertebrate embryo development, contributing to the formation of the embryonic mesoderm. Very little is known about the variability of plasma levels of Cripto in humans: only one study described higher levels of Cripto in patients with colon and breast carcinomas. Nevertheless the genetic contribution underlying this variability remains still unknown.
We measured the Cripto plasma levels in three isolated villages from Cilento area in South Italy (N=2026). The estimated heritability was very high (0.90). To identify genetic variants influencing the levels of Cripto, we performed both linkage and genome-wide association analyses. Through the linkage analysis a strong signal on chromosome 3p21.33 was detected (LOD=6.11). This locus exactly corresponds to the location of the Cripto gene. This result was supported by the genome-wide association analysis: in fact, several SNPs in this region were strongly associated with Cripto. The most associated SNP, located 1.5 kb upstream the Cripto gene has a p-value=6.9e-74; the C allele is associated with higher levels of Cripto (CC= 673.8±29.0 pg/ml; CT= 310.5±6.9 pg/ml; TT= 46.7±4.4 pg/ml). A linkage analysis conditional on the most associated SNP resulted in a complete loss

of the peak on the chromosome 3, showing that this SNP is able to explain the entire signal.
Further studies are required to identify the causal variant for Cripto variability.

P09.072
Oxidative damage of mtDNA in patients with Crohn's disease

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Crohn's disease (CD) is a nonspecific chronic inflammation of the gastrointestinal tract with an onset in the second to fourth decade of life. It is together with ulcerative colitis (UC) one of the two main inflammatory bowel diseases (IBD). The highest incidence of CD is in North America and Northern Europe. The etiology and molecular pathogenesis of CD is unknown but causes include genetic and environmental factors leading to defects in immunity and oxidative stress. Mitochondria produce large amounts of free radicals and have a low antioxidative status, therefore, mitochondrial DNA (mtDNA) is at high risk of oxidative damage. Furthermore, mitochondria play a crucial role in the autoimmune response, apoptosis and autophagy, all of which are implicated in the pathogenesis of CD. The aim of the study is to compare the common 4977bp deletion and fragmentation of mtDNA in CD patients and controls. We hypothesize that the frequency of the mtDNA deletion and fragmentation depends on patient's genotype. Two polymorphisms in the candidate genes for CD associated with oxidative stress (*NCF4*) and autophagy (*ATG16L1*) were selected. Testing of this hypothesis could shed light on CD pathogenesis with regard to the potential application of a supportive antioxidant therapy.

P09.073
CYP2C19 polymorphism and antidepressant response to Citaloperam in Iranian patients with major depression

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Introduction: Personalized drug therapy based on genotype analysis seems to be a promising approach to reduce adverse effects and enhance drug efficacy. The polymorphic expression of cytochrome P4502C19 (CYP2C19) is known to be one of the determinants responsible for the pronounced interethnic and interindividual differences in response and disposition of clinically important drugs such as citaloperam .On the basis of their ability to metabolize (S)-mephenytoin or other CYP2C19 substrates, individuals can be classified as extensive metabolizers (EMs) or poor metabolizers (PMs). CYP2C19*2 and CYP2C19*3 have been shown as the main polymorphisms contributing to the PM phenotype. This study aimed evaluating the CYP2C19 polymorphism and antidepressant response to Citaloperam in Iranian major depressive population
Methods: 77 patients with major depression were selected according to Hamilton test and then Citaloperam treatment was started. CYP2C19 polymorphism analyses were performed using a Restriction fragment length polymorphism (RFLP) method.
Results and Conclusion: Eight individuals carried two mutated alleles, being homozygous for CYP2C19*2, and thus could be classified as poor metabolizers. Whereas twelve subjects carried one mutated allele (CYP2C19*1/*2) of CYP2C19*2. For CYP2C19*3, we found three subjects with one mutated allele. The rest of subjects have the wild-type alleles that referred to as *1. It seems that there was no sginificant difference in the frequency of CYP2C19 allelic variants between the present study and other studies evaluating this.

P09.074
APOE and CYP2D6 in CNS Disorders

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The APOE and CYP2D6 genes cooperate in the pathogenesis and pharmacogenomics of some CNS disorders as well as in lipid metabolism. The distribution and frequency of polymorphic variants of APOE and CYP2D6 have been studied in the Spanish population, including control subjects (N=315), and patients with anxiety (ANX, N=285), depression (DEP, N=419), psychosis (PSY, N=162), stroke (STR, N=67), Alzheimer's disease (AD, N=231), Parkinson's disease (PAR, N=73), attention-deficit hyperactivity disorder (ADHA, N=42), migraine (MIG, N=217), epilepsy (EPI, N=71), vascular dementia (VD, N=198), vascular encephalopathy (VE, N=380), multiple sclerosis (MS, N=21), cerebrovascular insufficiency (CVI, N?238), brain tumors (BT, N=11), cranial nerve neuropathy (CNN, N=25), mental retardation (MR, N=115), and post-traumatic brain injury (PTBS, N=59). In the control population APOE variants were: APOE-2/2, 0.32%; APOE-2/3, 7.3%; APOE-2/4, 1.27%; APOE-3/3, 71.11%; APOE-3/4, 18.41%; and APOE-4/4, 1.59%. A clear accumulation of APOE-4/4 carriers was observed in AD (6.06%, p<0.001) and VD patients (6.57%, p<0.001). CYP2D6 extensive metabolizers (EM) accounted for 55.71% of the control population; intermediate metabolizers (IM) were 34.7%; poor metabolizers (PM), 2.28%; and ultrarapid metabolizers (UM), 7.31%. Significant differences in CYP2D6 genotypes were observed between DEP vs controls (p<0.02), vs PSY (p<0.02), vs PAR (p<0.05), vs BT (p<0.01); STR vs BT (p<0.05); BT vs Controls (p<0.05); and CNN vs controls (p<0.05). APOE-4 is a major risk factor for AD and VD. The accumulation of CYP2D6-PMs and UMs in different CNS pathologies may influence the therapeutic response to psychotropic drugs.
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P09.075
Role of CYP1A1 (T6235C) polymorphism and cigarette smoking in the development of coronary heart disease in the Tunisian population

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Aims: Cytochrome P450 1A1 (CYP1A1) plays an important role in maintaining the homeostasis of the cardiovascular system. This enzyme catalyses the formation and/or metabolism of several endogenous molecules such as arachidonic acid leading to the formation of cardioprotective metabolites. Furthermore, *CYP1A1* activates tobacco mutagens involved in carcinogenesis, atherogenesis, and teratogenesis through DNA adducts formation. This study aimed to evaluate the association between *CYP1A1* (T6235C) polymorphism and the risk of developing coronary heart disease (CHD) in the Tunisian population, and to assess the possible effect of the smoking habit on this association.
Methods: This case-control study consisted of 401 controls and 382 patients with CHD including 264 subjects with myocardial infarction (MI) and 118 with angina pectoris (AP). The genotyping of *CYP1A1* (T6235C) polymorphism was carried out by polymerase chain reaction (PCR) and restriction enzyme digestion.
Results: No significant association was found between *CYP1A1* (T6235C) polymorphism and CHD, MI or AP. However, the CC homozygous subjects may have a lower risk of developing CHD or MI (odds ratio (OR) 0.53; 95% confidence interval (CI) [0.16-1.79] and 0.20; 95% CI [0.02-1.57] respectively) when compared to TT carriers. This did not exceed the significance threshold. When considering the tobacco smoking status, the risk of CHD or MI decreased for the CC genotype carriers even after adjustment for potential confounders but without exceeding the significance threshold.
Conclusion : *CYP1A1* (T6235C) polymorphism does not seem to modify the risk of CHD, MI or AP in the Tunisian population even when considering the tobacco smoking habit.

P09.076
The functional polymorphism of (A-164C) of CYP1A2 gene increases the risk of angina pectoris but not the risk of myocardial infarction in the Tunisian population

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Background: Cytochrome P450 1A2 (CYP1A2) enzyme plays an important role in the homeostasis of the cardiovascular system through the metabolism of endogenous molecules such as arachidonic acids which have cardioprotective effects and exogenous molecules like tobacco chemicals that have atherogenic effects. The purpose of this study was to evaluate the association between *CYP1A2* (A-164C) polymorphism and coronary heart disease (CHD) in the Tunisian population and to determine whether smoking habit modifies this association.

Methods: we recruited 400 controls and 379 coronary heart disease patients including 261 subjects with myocardial infarction (MI) and 118 with angina pectoris (AP). The genotyping of *CYP1A2* (A-164C) polymorphism was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism.

Results: The results did not show any significant association between *CYP1A2* (A-164C) polymorphism and CHD and MI. However, the homozygous genotype CC of the single nucleotide polymorphism (SNP) A-164C was found to be significantly associated with AP (odds ratio (OR) 2.13; 95% confidence interval (CI) [1.20-3.77] p=0.01). We also, found that smokers carrying CC genotype have an increased risk of AP (OR 3.82; 95% CI [1.55-9.39] p=0.004) in comparison to those carrying AA genotype. After adjustment for traditional risk factors, the CC genotype was significantly associated with an increased risk of AP with or without considering the smoking status (OR 7.99; 95% CI [2.24-28.43] p=0.001)/(OR 3.29; 95% CI [1.45-7.84] p=0.004) respectively.

Conclusion: Our findings suggest that the CC genotype of *CYP1A2* (A-164C) polymorphism was associated with AP in the Tunisian population This risk increased significantly among smokers.

P09.077
Genetic markers of inflammation in patients with cardiovascular disease

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Essential hypertension (EH) is a common disease with severe cardiovascular complications (myocardial infarction and stroke), the major cause of mortality and morbidity in the modern societies. Blood pressure is a complex trait with multiple genetic contributors. Inflammation is the core process in pathophysiology of EH, and cytokines are biomediators that are involved in each stage of its development.

Our study was aimed to investigate an association between cytokine genes variants and cardiovascular disease, and to study their expression profile in leucocytes of patients with EH.

DNA and RNA samples were obtained from 364 patients with EH and 273 control subjects. 95 patients with EH had verified myocardial infarction, 45 - ischaemic stroke, and 219 had EH without cardiovascular complications. In our experiment, 11 SNPs in 10 genes analyzed, and an association study between EH patients and control subjects was performed. 84 cytokines and cytokine receptors genes were screened for their expression profile with the RT2ProfilerTM PCR Array (SuperArray Bioscience Corporation, USA).

An association was detected between IL1B T511C (P=0.029) and IL10 C627A (P=0.016) SNPs and EH. IL1B, IL6, IL10, IL12B and TNFA gene variants were found to be associated with cardiovascular complications of EH. Evidence for association with stroke was observed for IL1B T511C (P=0.022), IL-6 G572C (P=0.03), IL12B A1159C (P=0.017) and TNFA G308A. Patients with EH were demonstrated to have altered transcriptional activity of 21 cytokine genes. Our findings suggest that alterations in the genes encoding inflammatory components may have an input to the increased susceptibility to cardiovascular disease.

P09.078
GJB2 35delG and Mitochondrial A1555G mutations and etiology of deafness at the Gelibolu School for the Deaf in Turkey

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Objective: 35delG mutation in GJB2 (gap junction protein beta 2, connexin 26) gene is the most frequent mutation in patients with non-syndromic autosomal recessive deafness. The A155G mutation in the mitochondrial 12S rRNA is another important genetic alteration, which is associated with aminoglycoside-induced deafness. The aim of this study was to explore the etiology of deafness and the prevalence of both mutations in study cases.

Methods: We examined audiological and dysmorphological features of all children at Gelibolu School for the deaf. A questionnaire investigating prenatal, perinatal and postnatal etiological causes of deafness was prepared, and pedigree analysis was performed for each individual. After ENT examination, audiological tests and mutation analysis with RT PCR method were carried out.

Results: The GJB2 35delG and mitochondrial A1555G mutation was detected in 12% and was10% of all deaf school children, respectively. The percentages of genetic, acquired, both genetic and environmental and unknown etiologies were 62.5, 20.3, 15.6 and 1.6, respectively. One patient had both Waardenburg Syndrome and mitochondrial A1555G mutation, and one patient carried both 35delG and mitochondrial A1555G mutations. Interestingly, one sporadic case, was who developed deafness after fever and aminoglycoside treatment found to have homozygous 35delG mutation. His parents and healthy brother were heterozygous for the mutation.

Conclusion: Our results showed that dysmorphologic examination and mutation analysis are important for clarification of etiology, and thay can be helpful for genetic counselling.

P09.079
Genetic association of adipnectin with type 2 diabetes in Jordanian Arab population

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Adiponectin, a protein exclusively secreted by adipose tissue but present at low levels in obese individuals, is now widely recognized as a key determinant of insulin sensitivity and of protection against obesity-associated metabolic syndrome. In Jordan, prevalence of diabetes increased from 13.0% to 17.1% over a period of 10 years, which is twice the prevalence of Diabetes in the US 7.8%. In this study, we examined the contribution of the promoter variant rs266729 (-11377C>G) of ADIPOQ gene as a risk factor for diabetic patients in Jordan. DNA was extracted from blood samples for patients and controls .Polymerase chain reaction and restriction fragment length polymorphism (RFLP) were used to genotype this variant. A total of 420 type 2 diabetic patients and 230 controls were successfully genotyped. The results showed a significant genotypic (p=0.00001) and allelic (p=0.01) association with variant in the diabetic patients as compared to controls. This suggests that ADIPOQ gene plays a role in increasing the risk of diabetes, at least in the Jordanian Arab population.

P09.080
Gene expression analysis in type 1 diabetes prediction

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Background: Type 1 Diabetes (T1D) is considered to be a Th(helper)-1 autoimmune disease but T1D pathogenesis is probably more complex. For the efficient T1D prediction we still lack the sufficient tool for the autoreactive T cell detection. In this study we analysed by gene

expression array the effect of diabetes associated autoantigens on peripheral mononuclear cells (PBMC) with the purpose to identify promising (pre)diabetes cell biomarkers.
Methods: Twelve recent onset T1D patients, 25 their first degree relatives (9/25 autoantibody positive) and 13 healthy controls were tested. Their PBMC were stimulated by the cocktail of diabetes associated autoantigens (proinsulin, IA2 and GAD65 derived peptides). After 72 hours the gene expression was evaluated by high density gene microarray and the data were analysed with the emphasis on search for differences in the cell cascades activation.
Results: The highest number of differences was observed when relatives were compared to controls (69 pathways). Out of these 69 significant pathways 15% of them belong to "immune response pathways". The highest number of significant differences in immune response related pathways was observed when T1D patients were compared to healthy controls (24%). The important seems to be mainly the activation of Th17 and TGF-beta related cell processes.
Conclusions: The important differences were observed when the activation of cell processes after the exposure to diabetes related autoantigens was compared within T1D patients, the first degree relatives and controls. Genes involved in Th17 and TGF-beta cascades represent promising (pre)diabetes biomarkers. Supported by Czech Ministry of Education (NPVII2B06019).

P09.081
Association of *NOS3* with Diabetic Retinopathy in the Azorean population

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Diabetic Retinopathy (DR) is an ocular manifestation which affects up to 80% of all patients who have had diabetes mellitus (DM) for 10 or more years. It has been suggested that, in diabetes, the increased level of Nitric Oxide in the retina, causes oxidative stress and subsequent pathological changes resulting in DR. Two enzymes which can cause vascular oxidative stress - the ecNOS and ACE - may have important roles in DR.
The objective of this study was to identify possible associations between DR and *NOS3* (T-786C and 4a/4b) and *ACE* (I/D) polymorphisms. 137 DM patients from the Azores were investigated: 76 DM patients with DR were compared with 61 DM controls without DR. *ACE* I/D polymorphism was typed by PCR-RFLP. *NOS3* polymorphisms 4a/4b and T786C were typed by PCR and PCR-RFLP, respectively. Linkage disequilibrium (LD) between *NOS3* polymorphisms was analyzed using Arlequin 3.1.
Allelic frequencies for the *NOS3* and *ACE* polymorphisms did not differ between both groups. All polymorphisms were in Hardy-Weinberg equilibrium with the exception of *ACE* I/D polymorphism in diabetic men (without DR). This HW disequilibrium suggests an association of I/D polymorphism to DM. One *NOS3* haplotype (bbCC) was statistically significant associated to DR (p=0,03;OR=3,625). These polymorphisms were not in LD.
This study identified a clear association of *NOS3* to DR. After testing this association, in a larger sample, this susceptibility haplotype can be used as a molecular marker for DR in DM patients. The sex-specific association of *ACE* to DM needs to be further investigated.

P09.082
Genes, viruses and inflammation in idiopathic (dilated) cardiomyopathy

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Background: dilated cardiomyopathy (DCM) is characterized by impaired contractility and ventricular dilatation . Mutations in the currently known DCM-genes are only found in a small number of DCM patients and the clinical phenotype in families with a known DCM mutation varies considerably. Cardiotrophic viruses and/or inflammation also play an important role in DCM and could explain interindivuals clinical variability. We assessed the presence of genetic variants and cardiac viruses/ inflammation in a group of 30 patients with an idiopathic DCM.
Methods: genetic analysis was performed on DNA through CardioCHIP analysis, a CHIP-based high throughput resequencing platform, simultaneously analysing 34 genes involved in inherited cardiomyopathies. The presence of cardiotrophic viruses and/or inflammation was assessed on cardiac biopsies.
Results: 16 of the 30 patients (52%) had genetic variations at CardioCHIP analyses. Cardiac biopsy in these 30 patients revealed ParvoB19 virus infection (>500 copies per mcg isolated DNA) in 7 patients, inflammation (>14 CD45-cells/mm^2) in 4 patients and active myocarditis in 1 patient. Of these 12 patients with viral infection and/ or inflammation (12/30=40%), 6 patients also had genetic alterations (6/12=50%) on cardioCHIP analysis. Family history was positive for sudden death in 3 of these 6 patients. We are currently performing DNA-analysis combined with cardiac biopsies in additional patients.
Conclusion: although based on small numbers, these data demonstrate that viral or inflammatory components as a cause for DCM do not exclude a genetic component and vice versa. These data are important for genetic counseling of individuals with DCM.

P09.083
Identifying genes predisposing Great Danes and Newfoundland dogs to dilated cardiomyopathy

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Recent bottlenecks at breed creation have led to high levels of inbreeding and linkage disequilibrium within dog breeds, making dogs perfect models for genome-wide association (GWA) mapping of genetic risk factors for inherited diseases.
Dilated cardiomyopathy (DCM) of non-ischemic origin is a heart muscle disease characterized by reduced myocardial contractile function, dilation of cardiac cavities and thinning of the cardiac walls, which may lead to congestive heart failure, arrhythmias or sudden cardiac death. The disease has been shown to be familial in dogs and phenotypically identical to human DCM.
Two dog breeds predisposed to DCM, Great Danes and Newfoundland, were used in this case-control study to identify genetic risk factors predisposing to DCM. Both healthy and affected dogs were thoroughly examined by veterinary cardiologists and characterized using echocardiography. A proportion of dogs also underwent post-mortem histological examination, to confirm or refute the diagnosis of DCM.
GWA study identified different regions of significant association in each breed, indicating different genetic risk factors. Identifying DCM genes in dogs will contribute to identification of causative genes for the corresponding human disease, and lead to advantages for both canine and human health.
- 1 -

P09.084
New susceptibility loci for dilated cardiomyopathy in Irish Wolfhounds

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Dilated cardiomyopathy (DCM) is a progressive myocardial disease in human and dogs. The disorder is associated with cardiac dilation and reduced systolic function. In human, more than 25 genes are reported whose mutations are related to DCM but they still explain

only a minor part of familial DCM in human. Moreover, many of theses genes were excluded being involved in pathogenesis of DCM in dog breeds. Therefore, identifying susceptibility loci in dogs might identify new DCM causing genes in human. In addition, dogs are excellent models for new therapy concepts e.g. gene therapy which already could be demonstrated.
Irish Wolfhounds show a high prevalence for DCM. In this dog breed, it is a familial adult-onset disease with a dominant major gene model including a sex dependent allele effect.
Here, we present a whole genome linkage analysis using Illumina canine HD beadchip. The linkage data set included 71 dogs from 11 families. Overall, 47 dogs were DCM affected und 24 DCM unaffected. Multipoint linkage and haplotype analysis for SNP sets with different SNP densities were performed as well as non-parametric quantitative trait approaches with Merlin version 1.1.2. Consistent regions for Zmean and LOD score were identified on dog chromosomes 7, 8, 10 and 13. For some regions we identified candidate genes.

P09.085
Intron 3 VNTR polymorhism in XRCC4 DNA repair gene is associated with rehumatoid arthritis in Turkey

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Objectives of the study: DNA repair genes are involved in several disease such as cancers, autoimmune diseases etc. Rheumatoid arthritis (RA) is a chronic, inflammatory disease that affects 0.5-1.0 % of the adult population. It is estimated, that at least 50 % of the risk of developing RA is determined by genetic factors. In this study, we investigated whether four polymorphisms in DNA repair genes (*XPD*, *XRCC1* and *XRCC4*) are associated with RA.
Methods: Sixty-five patients with RA and 70 healthy controls were examined for *XPD* (A-751G), *XRCC1* (A399G) and *XRCC4* (intron 3 VNTR and G-1394T) polymorphisms. All polymorphisms were genotyped by PCR and/or PCR-RFLP and analyzed statistically.
Results: The intron3 VNTR polymorphism in *XRCC4* showed an association with RA. The distribution of DD, DI and II genotypes for the gene was 20%, 33.8% and 46.2% in RA patients compared with 14.3%, 60% and 25.7% in the controls. The DI genotype was found at lower frequency in RA patients (χ^2=8.227 p=0.0021), while the II genotype was more frequent in RA patients (χ^2=5.285 p=0.010). For both the intron 3 VNTR and G-1394T polymorphisms in the XRCC4 gene region the observed genotype counts deviated from those expected under Hardy-Weinberg equilibrium (p=0.02 and 0.004, respectively), but there was no deviation for other gene polymorphisms. There is no statistical difference between the RA patients and control groups for *XPD* (A-751G), *XRCC1* (A399G), and *XRCC4* (G-1394T) gene polymorphisms (p>0.05).
Conclusion: Our results suggest that the intron 3 VNTR polymorphism in the *XRCC4* gene may be associated with RA ethiopathogenesis.

P09.086***
Targeted re-sequencing of candidate genes for developmental dyslexia: towards the discovery of novel single nucleotide polymorphisms

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Current next-generation sequencing platforms are well adapted for high-throughput analysis of sequence variants of many genes in parallel. For this pilot project, we aimed to identify novel single nucleotide polymorphisms (SNPs) and to assess the frequencies of known SNPs in 11 candidate genes for dyslexia, a common childhood learning disability with a large genetic component. Using a pooling strategy, 100 dyslectics of Finnish origin were combined into 10 equal pools. In addition, we used three HapMap DNA samples for methodological quality control. Target regions, including protein-coding exons surrounded by at least 50bp intronic sequence, and UTRs were enriched using the Halo Genomics selector probe technology with average design coverage of 95.9% and amplicon size of 420kb for all regions. Each pool was indexed with a unique barcode whereas the HapMap samples were indexed individually before paired-end sequencing on a SOLiD 4 platform, at Science for Life Laboratory, Stockholm, Sweden.
This approach allowed us to perform deep sequencing in order to detect novel variants with an estimated theoretical coverage of 30-300X per base. Analysis of the preliminary data shows a 99% SNP concordance for the HapMap DNA used in this project. Presently, SNP calling using different software solutions is underway and a panel of SNPs will be verified using the Sequenom MassARRAY iPLEX Gold genotyping methodology.
In summary, we describe a cost-effective approach for assessing the frequencies of sequence variants in selected gene regions using pooled samples of DNA.

P09.087
Genetic overlap of airway obstruction and emphysema

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Airway obstruction and emphysema are two features of chronic obstructive pulmonary disease (COPD). COPD patients can display one phenotype or both at one time. We used the Nelson cohort comprising ~3000 individuals with lung function measurements and CT-scans to determine genes that contribute to both features. We performed genome-wide association studies on both sub-phenotypes. Airway obstruction was investigated in a case-control design (1030 cases with FEV1/FVC<0.7, 953 controls with FEV1/FVC>0.7 and FEV1 >90%pred, both groups being heavy-smokers with >20 pack-years, and 846 blood bank controls). Emphysema was investigated as a quantitative trait. To account for center-derived differences in these measurements we used 15th percentile (p15) of density distribution adjusted for air density in the trachea. p15 was analyzed using linear regression adjusting for age and pack-years smoking in 3047 subjects. To find overlap between these two sub-phenotypes we selected all SNPs with p<0.001 in each analysis, yielding a total of nine SNPs corresponding to four genes. When these genes were investigated in GeneMania they were enriched with an additional 9 genes directly interacting or co-expressed/co-localized with query genes and two of them point to a drug resistance pathway (GATHER p<0.0001, Bayes factor 6).
This is an interesting approach that can help identifying a shared etiology of two distinct sub-phenotypes of a single complex disease.

P09.088
HIF1A, PPARG and PPARGC1B gene variants and physical performance in middle school-age children

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It has long been recognized that the interindividual variability of physical performance traits and the ability to become an elite athlete have a strong genetic basis. The genetic loci that influence these phenotypes are now being sought. At present, there are few studies related to the search of the associations of gene polymorphisms with physical, physiological and skill parameters in children and adolescents. The aim of the study was to determine the association between HIF1A Pro582Ser, PPARG Pro12Ala, PPARGC1B Ala203Pro gene polymorphisms and performance-related traits in 455 middle school-age children. The assessment of physical performance was conducted with a number of physical and physiological tests. Gene variants were determined by PCR. The discovered correlations concerned primarily sprint performance traits, being in agreement with the generally accepted data: the HIF1A 582Ser, PPARG 12Ala and PPARGC1B 203Pro alleles were associated with the maximal values of standing long-jump and handgrip strength, whereas the HIF1A Pro582, and PPARGC1B 203Pro alleles correlated with the ability to

perform endurance performance (1-min bench trunk-curl testing of abdominal muscular endurance). In conclusion, HIF1A Pro582Ser, PPARG Pro12Ala, PPARGC1B Ala203Pro gene variants are strongly associated with several performance-related traits in physically active middle school-age children.

P09.089
Association between polymorphisms of mannose-binding lectin and Epstein-Barr virus nuclear antigen 1 IgG antibody level

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Increasing data support that infection by the Epstein-Barr virus (EBV) can be considered as a risk factor for multiple sclerosis (MS). Many studies have suggested a correlation between elevated EBV nuclear antigen 1 IgG (EBNA-1) level, MS and its disease activity. However, scarce data are available on the regulation of EBNA-1 level after EBV infection.

Hence, we decided to follow annual changes of EBNA-1 level in hereditary angioedema patients (as annual control samples of these patients were available for the last seven years). EBNA-1 level was also measured in healthy (N=182) and MS individuals (N=96) in order to analyze its correlation with exon 1 polymorphisms of the mannose-binding lectin (MBL) gene. EBNA-1 level was determined by ELISA; A/0 polymorphisms of MBL were genotyped by real-time PCR.

For the studied period EBNA-1 IgG levels were almost constant in each individual. Lower EBNA-1 IgG level was found in each studied group in carriers of a genotype (0/0) causing functional deficiency of MBL. By summing up data of all the 376 participants we found a significant difference (p=0.043) in the level of EBNA-1 between carriers of the 0/0 genotype (median 100.0 U/ml (IQ range 25.1-243.1)) and non-carriers (192.0 (90.3-421.4), respectively).

Our data indicate that EBNA-1 IgG antibody titer is relatively stable for years denoting a role of genetic factors in the regulation of this antibody. Polymorphisms of MBL may - at least partially - determine EBNA-1 level both in healthy and MS patients.

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P09.090
Comprehensive study of genetic factors determining the development of rheumatic autoimmune diseases in humans.

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The purpose of our research was to find genetic factors of autoimmune arthropathies. During this research comparative genotyping of two Russian cohorts was performed, both of which combine HLA-B27 positive individuals.

Among a large number of MHC genes it was the HLA-B27, which was strongly associated with autoimmune arthropathies. Also few other candidate genes were involved in the development of pathogenesis. Most of those genes take part in various immunological processes such as differentiation and antigen presentation. In this research we study variability of candidate gene - ERAP1 specially in Russian Caucasian population.

We analyzed an association of several most associated with AS coding-region SNPs in the ERAP1 gene. Was genotyped 5 ERAP1 SNPs (rs2287987, rs30187, rs10050860, rs17482078, rs27044) in 70 AS patients and 83 HLA-B27-positive healthy controls from the Russian Caucasian population by SSP-PCR.

Considerable differences in allele's frequencies within patients vs control cohort were shown for 3 of 5 SNPs under investigation. Using the EM-algorhitm we reconstructed 3-marker haplotypes that distinguish with high probability two cohorts due to differences in the haplotypes frequencies. In such a way both the sensitive, CCT, haplotype and the protective, TTC, one were predicted. To verify the calculation we determined genuine frequencies of 5-marker haplotypes in AS cohort by haplotyping of individual cDNA samples. As a result presumably risk CCT haplotypes of ERAP1 gene was detected in 65 of 69 examined AS patients' genomes.

We forecasted ERAP1 risk haplotype CCT (rs17482078/10050860/2287987) and demonstrated that its frequency detected within AS cohort reaches 88%.

P09.091
Polymorphisms in the factor VII gene and the risk of myocardial infarction in patients with coronary thrombosis: A hospital based study

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Factor VII (FVII) is one of the central proteins in the coagulation cascade. High plasma levels of coagulation FVII have been suggested to be the predictors of death due to coronary artery disease. Since polymorphisms in the FVII gene contribute to variations in FVII levels and such polymorphisms may be associated with the risk of myocardial infarction(MI), which is precipitated by thrombosis. We studied a total of 319 patients, 152 of whom had severe angiographically documented for coronary atherosclerosis and no documentation of a previous MI. As a control group, 167 patients with normal coronary arteriograms were also included as healthy controls. We measured the levels of activated FVII and assessed three polymorphisms in the FVII gene, one involving the promoter (*A1*and *A2* alleles), one involving the catalytic region (R353Q), and one involving intron 7. Each of the polymorphisms influenced the FVII levels. To emphasize the contribution of genetic factors, we studied patients with a family history of thrombosis. Patients with the *A2A2* and *QQ* genotypes had the lowest levels of activated FVII (56% and 70 % lower, than the levels in patients with the wild-type genotypes). In the patient group, there were significantly more heterozygotes and homozygotes for the *A2* and *Q* alleles among those who had not had a MI than among those who had an infarction (P=0.007) for the presence of the promoter polymorphism and P=0.01 for the presence of the R353Q polymorphism. Our findings suggest that, certain FVII genotypes have a role in protection against MI.

P09.092
Modeling Parkinson's disease in rats

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SAGE Labs has created targeted, homozygous knockouts of Lrrk2, Park2 (Parkin), Park6 (Pink1) and park7 (DJ-1) in rats. The Lrrk2 gene encodes the leucine-rich repeat kinase 2. Lrrk2 mutations account for 5-6% of familial Parkinson's dieases and 1-3% in sporadic PD. Collectively, these mutations result in the most common cause of PD. The Park2 gene, Parkinson disease (autosomal recessive, juvenile) 2, encodes the protein Parkin that localizes to the cytoplasm of neurons and mediates the degradation of improperly folded proteins. Roughly 20% of patients with Parkinson's disease onset before age 40 have mutations within Park2. Park7 (Parkinson disease [autosomal recessive, early onset] 7) gene, synonym DJ-1, belongs to the peptidase C56 family of proteins. In humans, loss of function of Park7 leads to a form of early-onset Parkinson's disease. Pink1 (PTEN-induced putative kinase 1) specifies a serine/threonine protein kinase. Mutations in Pink1 are implicated in early-onset Parkinson's disease. The Pink1 model is being developed in collaboration with the Michael J. Fox Foundation.

The targeted genomic editing for all theses genes was performed using the Zinc Finger Nuclease (ZFN) technology. The availability of genetically modified rat models for Parkinson's disease has opened up new avenues for researchers to better understand the pathophysiology of the disease and explore novel therapeutic avenues for its treatment.

P09.093
A polymorphic miR-155 binding site in AGTR1 is associated with cardiac hypertrophy in patients with Friedreich ataxia

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Background: Friedreich ataxia (FRDA) is a neurodegenerative condition with a heterogeneous cardiac phenotype caused by an expanded GAA trinucleotide repeat in the *FXN* gene. The number of GAA repeats on the smaller *FXN* allele (GAA1) only accounts for a portion of the observed variability in cardiac phenotype. Genetic modifying factors, such as single nucleotide polymorphisms (SNPs) in the Renin-Angiotensin-Aldosterone system (RAAS), may contribute to phenotype variability. This study investigated variability in the angiotensin-II type-1 receptor (*AGTR1*), angiotensin-converting enzyme (*ACE*), and *ACE2* genes as cardiac phenotype modifying factors in FRDA.

Methods: Review of the *AGTR1*, *ACE* and *ACE2* genes identified twelve haplotype tagging SNPs. Correlation of these SNPs with left-ventricular septal wall thickness (SWT), relative wall thickness, left-ventricular end-diastolic volume index and left-ventricular mass index (LVMI) was examined in a large Australian FRDA cohort (n=79) with adjustments performed for GAA1, sex and systolic blood pressure.

Results: The *AGTR1* polymorphism rs5186 was more common in FRDA patients than in a control population (minor allele frequency: 0.35±0.04 versus 0.22±0.02; p=0.002). Using a recessive model of inheritance, the C allele of rs5186 showed a significant trend with increased SWT (AA+AC=10.7±2.1mm, CC=11.7±2.8mm; p=0.005) and LVMI (AA+AC=99.7±28.6g/m^2, CC=119.6±34.0g/m^2; p=0.001). rs5186 increases expression of *AGTR1* by altering the binding site for miR-155, a regulatory microRNA. No associations were observed for the remaining RAAS polymorphisms.

Conclusions: rs5186 had an increased frequency in a large FRDA population and associated with increased SWT and LVMI. This study supports the role of RAAS polymorphisms as modifiers of cardiac phenotype in FRDA.

P09.094
The common polymorphism of FTO gene is associated with overweight or obesity in Russians

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The common rs9939609 A/T polymorphism of the fat mass and obesity-associated gene (FTO) was identified as an obesity-susceptibility gene variant by several independent large-scale genome association studies. The aim of the study was to investigate FTO gene variant with overweight or obesity in Russian population. PCR-restriction fragment length polymorphism was used to determine the rs9939609 polymorphism in 42 patients with overweight or obesity and 135 healthy controls (BMI less than 25 kg/m2) (all Caucasians). The genotype distributions of both groups were in the Hardy-Weinberg equilibrium. The frequencies of AA, AT and TT were 0.381, 0.333 and 0.286 in the overweight or obesity group, and 0.192, 0.489 and 0.319 in the controls (Chi-square = 6.62, P = 0.037). The frequency of AA genotype (with greatest risk for obesity) in case group was significantly higher than that in the controls (Chi-square = 6.28, P = 0.012). In conclusion, the AA genotype of FTO gene might be a risk factor of overweight or obesity in Russian population.

P09.095
A correlative study of genetic and epigenetic factors associated with cardiovascular pathology

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A correlative study of genetic and epigenetic factors associated with cardiovascular pathology

This work is presenting the results of a correlative study of genetic, epigenetic and metabolic factors associated with cardiovascular pathology diagnosed in 100 elderly vs 100 healthy (25 young, 25 adult and 50 aged) persons. HPLC estimation of methylome metabolites and PCR assays of gene polymorphisms as well as methylation sensible restriction estimation of global DNA methylation level associated with the classical karyotyping in limphocyte cultures derived from peripheral blood have been performed. The results showed interesting correlations between the modification of normal S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH) ratio towards the increased level of SAH and decreased level of SAM, a pronounced hypomethylation of global DNA. These metabolites levels and epigenetic factors were correlated with genetic aspects in terms of an altered karyotype, as well as high frequency of polymorphism in genes controlling the methylome (methylenetetrahydrofolatereductase gene, *mthfr*) and hypertension (angiotensin-catabolysing gene, *ace*). The study also included the investigation of the above parameters variation when persons took a 3 months inulin containing diet. The results demonstrated that an altered metabolites level (increased SAH concomitant with a lowered SAM concentrations) determined a global DNA hypomethylation and hypercholesterolaemia. The genetic and epigenetic factors are well correlated with a high frequency of abnormal allelic expression of the *mthfr* and *ace* genes.The metabolomic parameters showed an interesting variation towards the decrease of SAH and increase of SAM concentration after three months of the inulin enriched diet.

P09.096
Using genetic risk models to identify high-risk groups for the prevention of multifactorial diseases: will it work?

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Purpose: Genetic risk models could be useful in identifying high-risk groups for the prevention of multifactorial diseases. We investigated epidemiological parameters that may affect the performance of this risk stratification strategy.

Methods: Using simulated data, we assessed sensitivity, specificity, positive and negative predictive value for all possible thresholds of the risk model and investigated how these measures depend on the frequency of disease in the population, the frequency of the high-risk group, and the discriminative accuracy of the risk model, assessed by the area under the receiver-operating characteristic curve (AUC). We modeled genetic risk scores of 50 genes with equal odds ratios and genotype frequencies, which were varied across scenarios.

Results: We show that when the frequency of the high-risk group was lower than the disease frequency, positive predictive value increased with the AUC but sensitivity remained low. When the frequency of the high-risk group was higher than the disease frequency, sensitivity was high but positive predictive value remained low. When both frequencies were equal, both positive predictive value and sensitivity increased with increasing AUC, but higher AUC was needed to maximize both measures.

Conclusion: The performance of risk stratification is strongly determined by the frequency of the high-risk group relative to the frequency of disease in the population. The identification of high-risk groups with appreciable combinations of sensitivity and positive predictive value requires higher AUC.

P09.097
Two novel loci, EML4/COX7A2L/KCNG3 and *IRF4/EXOC2*, were identified to be associated with Graves' Disease in Han Chinese

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Graves's disease (GD) is a common autoimmune thyroid disease and displays a strong female predominance. To identify new susceptibility genes that increase the risk of GD in a Han Chinese population, we conducted a genome-wide association study for GD in 596 patients and 804 controls. We found two loci associated with GD, *EML4/COX7A2L/KCNG3* (P = 3.21 × 10^{-6}; odds ratio [OR] = 1.59, 95% confidence interval [CI] =1.31-1.93) and *IRF4/EXOC2* (P = 1.1 × 10^{-5}; allelic OR = 1.44, 95% CI = 1.22-1.69). Within these loci are biological candidate genes that influence self tolerance and the response to estrogen, which may provide a partial explanation as to why women are predisposed to GD. We also confirmed that *HLA* loci (P = 2.75 × 10^{-11}; OR = 2.02, 95% CI = 1.64-2.78) and *TSHR* (P = 1.77 × 10^{-6}; OR = 1.48, 95% CI = 1.26-1.74) were associated with GD risk. In the present study, the HLA-DQB1*0202, HLA-DRB1*0701, HLA-DRB1*1202, and HLA-DRB1*1602 were associated with GD; these HLA alleles were not previously reported to be associated with GD in other Asian or Caucasian populations. Our results may lead to a better understanding of the underlying mechanisms of GD pathogenesis and female-biased autoimmunity.

P09.098
Characterization of the Genomic Risk Profile in Cerebrovascular Diseases

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The genomic cerebrovascular panel proposed here deals with the study of genes involved in different events that trigger the atherogenic process, such as: lipid metabolism, endothelial function, immune response, and stability of atherosclerotic plaques (thrombosis).
A total of 20 genetic variants in 15 different genes related to atherogenesis have been determined in 457 individuals, 153 patients with vascular dementia (VaD), 148 with vascular encephalopathy (VaE), 46 stroke patients (ACV) and 110 control subjects without ischemic or hemorrhagic stroke history, or other cerebral diseases, aged over 50 years.
Significant differences between controls and stroke patients were found for APOE*2/3 (6.12% vs. 15.22%, p<0.05), APOE*4/4 (14.29% vs. 10.87%, p<0.05) and IL6*-573GG (29.70% vs. 38.71%, p<0.05). The most informative genotypes in vascular dementia were AGT*235TT (22.83% vs. 6.08%, p<0.001) that showed a protective effect, IL6*-573GG (29.70% vs. 68.29%, p<0.001) and IL6R*1510AA (25.74% vs. 45.12%, p<0.001). Relevant genotypes for VaE were IL6*-573GG (29.70% vs. 51.67%, p<0.001) and IL6R*1510AA (25.74% vs. 45.00%, p<0.001).
The results found in this preliminary study show the relationship between the plasmatic levels of cytokines and the major incidence of cerebral vascular accidents. Increased levels of IL6 and his receptor IL6R increase the recruitment of monocytes and macrophages to the lesion-prone sites contributing to the formation of the plate of atheroma. The potential of other related markers for increased risk to develop atherogenesis associated with lipid metabolism, hypertension or thrombosis, must to be reconsidered when are compared with the high informative capacity showed by genes related with inflammation response.

P09.099
Identification of a novel glaucoma locus in dogs

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Glaucoma is the second leading cause of blindness and a common eye disease in both human and dogs. Glaucoma is characterized by loss of retinal ganglion cells, damage of the optic nerve and by elevation of the intraocular pressure. Both primary and secondary glaucoma are diagnosed in both species, primary being considered a hereditary complex disease with multiple susceptibility genes combined with environmental factors. No canine glaucoma genes have been identified although the disease affects several breeds including Dandie Dinmont Terrier (DDT). In DDT primary glaucoma develops slowly affecting middle-aged dogs and presents abnormalities in the iridocorneal angle. To map the glaucoma locus, we genotyped 23 affected and 23 control DDTs using canine SNP arrays. The average age of onset in affected dogs was 7 years. Control dogs had been confirmed healthy over the age of 10 years. Association analysis mapped a single locus on CFA8 (P_{raw}=1.6*10^{-7}, P_{genome} =0.001) spanning a region of ~10 Mb. Fine-mapping with 146 additional samples and 111 additional SNPs on 100 kb average spacing narrowed the association to a 2 Mb haplotype with p-value of 1.63*10^{-10}. The identified locus is syntenic with a region associated with human glaucoma, although no genes within the region have implicated. Targeted re-sequencing of the 2 Mb region revealed several possible candidate variants that are may be causal for glaucoma in DDTs. Further analysis of the re-sequencing data will likely identify a novel candidate gene for human glaucoma and establish an important late-onset glaucoma model in a large animal model.

P09.100
CYP1B1 as a modifier gene for two Brazilian families with juvenile-onset open angle glaucoma harboring the C433R mutation in the MYOC gene.

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Glaucoma comprises a heterogeneous group of optic neuropathies characterized by accelerated loss of optic nerve ganglion cells, clinically evaluated as increased cup to disc ratio and elevated intraocular pressure (IOP), representing the world's leading cause of irreversible blindness. Mutations in the *MYOC* gene were associated with primary open angle glaucoma (POAG) and juvenile-onset open angle glaucoma (JOAG). In the Brazilian population, the C433R is the most common mutation related to POAG and JOAG. Some authors have suggested the participation of *CYP1B1* gene as a modifier of POAG phenotype in case/control studies. The objective of this study was to evaluate the association between variants of the *CYP1B1* gene and C433R mutation in the *MYOC* gene in modulating glaucomatous phenotype in a family based approach. The individuals of two Brazilian families with JOAG and POAG, harboring the C433R mutation in the *MYOC* gene with incomplete penetrance were clinically assessed and the coding regions of the *CYP1B1* gene were screened. The R48G, A119S, V432L, D449D, and N453S polymorphisms were observed in members from both families. The genotype/phenotype correlation was performed, but the relationship of these polymorphisms with age of onset, IOP and cup to disc ratio was not considered statistically significant. This result may indicate that the *CYP1B1* gene is not responsible for modulating the disease phenotype in these families.

P09.101
Uniparental disomy and progressive clonal selection: a common mechanism causing late onset β-thalassemia major?

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We present three cases of "severe late onset β-thalassemia" in three independent subjects, all presenting with the mild phenotype of beta thalassemia minor up to adult age and developing a severe transfusion dependent phenotype in the third and fourth decade of life when a presumed homozygosity for the β-thalassemia mutation is observed. In all three cases molecular analysis shows sequences in which homozygosity for the beta-thalassemia mutation occurs in the presence of a small but consistent wildtype signal in DNA extracted

from peripheral blood, from buccal mucose and from erythroid cultures. Loss of heterozygosity due to a deletion of one allele was excluded by MLPA analysis. Affymetrix SNP-array analysis reveals homozygosity for a large number of SNP's in a region on the short arm of chromosome 11 containing the beta-globin gene with a low background of wildtype SNP's, indicating mosaicism for a partial uniparental isodisomy of chromosome 11p.
Clonal selection for hematopoietic stem cells containing the uniparental isodisomy for the mutant beta-globin gene during life may account for the progressive development of the disease. A similar observation for a single case was made by Chang et al. (Haematologica 2008, 93(6):913-916), who found this in a single patient with late-onset β-thalassemia Major. Our study demonstrates that uniparental isodisomy of chromosome 11p15 is apparently more frequently associated with late-onset transfusion dependent β-thalassemia in presumed carriers at birth, representing a novel mechanism leading to this special form of beta-thalassemia and in other late-onset genetic diseases.

P09.102
Greyhound meningoencephalitis: Major Histocompatibility Complex association highlights a potential model for human neuroinflammatory disease.

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Neuroinflammation is common in humans and often has important and devastating consequences. In recent years there has been increased focus on the fact that neuroinflammation may underlie many neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. Furthermore, the predisposition of certain families to neuroinflammatory disorders such as Viliuisk encephalitis, Rasmussen's encephalitis and multiple sclerosis, highlights the potential of genetic predisposition to neuroinflammation.
We have recently identified a unique breed-associated encephalitis in young greyhounds. Although the underlying cause is unknown, the early onset and high frequency of disease in siblings makes a genetic predisposition likely. This disorder is invariably fatal and the unique pattern of distribution of inflammation within brain tissue made it possible to accurately phenotype this condition and allowed for the collection of diseased siblings, healthy siblings, parents and unrelated control tissues.
In preliminary studies, Major Histocompatibility Complex (MHC) Dog Leukocyte Antigen (DLA) three locus class II haplotypes were determined in 39 diseased dogs with post mortem confirmed disease, and 111 unrelated healthy controls. A significant association was detected between the DLA-DRB1*01802/DQA1*00101/DQB1*00802 haplotype and the development of disease, observed in 38.5% cases in contrast to 11.7% controls (OR=4.7, Chi2=11.9, p<0.0006).
A genome wide association study (GWAS) was performed using 25 unrelated diseased animals and 24 unrelated controls. No regions achieved genome wide significance but results from a larger cohort are pending.
The absence of a strong association in the GWAS study suggests this disease is not monogenic. The DLA association could reflect either an autoimmune or infectious cause.

P09.103
A genome-wide association study for two autoimmune diseases in Sardinia.

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Autoimmune diseases are extremely common in Sardinians, with the highest incidence worldwide observed for Multiple Sclerosis and Type 1 Diabetes. To detect novel loci associated with both diseases, we analyzed 1,922 controls, 2,280 Multiple Sclerosis (MS) and 1,377 Type 1 Diabetes (T1D) unrelated cases that were genotyped with the Affymetrix 6.0 array. We used Birdseed-v2 to perform genotype calling over a cluster containing all individuals and successively applied standard quality filters to each sample and marker. We then performed three Genome Wide Association Studies (GWAS): two for MS and T1D respectively, and one for shared loci using all available cases. Quality filters were re-applied to each specific data set, leading to a minimum of 471,016 markers available for association. For T1D, only the HLA locus reached genome-wide significance (p=4x10-108), but other known genes were confirmed at lower p-values, including IL2RA and CTLA4. For MS, we confirmed the association at HLA (p=4x10-31), IL2RA, CLEC16A, CD58 and CBLB. SNPs with borderline significance (P<5x10-6) on chr2q13 and chr8q21.12 with nearby strongly functional candidate genes suggest novel potential loci that require further analyses. Finally, the GWAS on shared autoimmunity confirmed the HLA locus and IL2RA gene, and revealed two possible candidates at borderline significance on chr9q21.33 and chr10q26.3. To increase the number of tested variants and to refine the association at known and potentially novel loci, we are performing imputation of un-typed markers using Sardinian and 1000 Genomes sequencing data, while the most associated markers are validated by de-novo genotyping in the entire cohort.

P09.104
Genetic architecture of circulating lipid levels

Genetic architecture of circulating lipid levels

Serum concentrations of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and total cholesterol (TC) are important heritable risk factors for cardiovascular disease. Although genome-wide association studies (GWAS) of circulating lipid levels have identified numerous loci, a substantial portion of the heritability of these traits remains unexplained. Evidence of unexplained genetic variance can be detected by combining multiple independent markers into additive genetic risk scores. Such polygenic scores, constructed using results from the ENGAGE Consortium GWAS on serum lipids, were applied to predict lipid levels in an independent population-based study, the Rotterdam Study-II (RS-II). We additionally tested for evidence of a shared genetic basis for different lipid phenotypes. Finally, the polygenic score approach was used to identify an alternative genome-wide significance threshold prior to pathway analysis and those results were compared to those based on the classical genome-wide significance threshold. Our study provides evidence suggesting that many loci influencing circulating lipid levels remain undiscovered. Cross-prediction models suggested a small overlap between the polygenic backgrounds involved in determining LDL-C, HDL-C and TG levels. Pathway analysis utilizing the best polygenic score for TC uncovered extra information compared to using only genome-wide significant loci. These results suggest that the genetic architecture of circulating lipids involves a number of undiscovered variants with very small effects, and that increasing GWAS sample sizes will enable the identification of novel variants that regulate lipid levels.

P09.105
A genome-wide association study of rheumatoid arthritis without antibodies against citrullinated peptides

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Rheumatoid Arthritis (RA) comprises different subsets based on the presence or absence of autoantibodies termed anti-citrullinated peptide antibodies (ACPA). This heterogeneity may reflect important biological differences underlying the disease process. Previous genome-wide association studies mainly addressed ACPA positive RA patients, and consequently detected genetic risk factors primarily associated with ACPA positive disease. The identified genetic factors are typically related to the immune system, pointing to an (auto)-immune disease mechanism in these patients. For ACPA negative RA little is understood about the genetic risk factors, although a substantial part of the disease risk is heritable in this group of patients.
Our goal was to investigate the genetic risk factors in ACPA negative RA. Therefore, we performed a large scale genome wide association study (GWAS) study in a Dutch cohort of 884 ACPA negative RA patients and 4011 controls, and an additional cohort of 699 Caucasian patients and 1653 controls with different nationalities. All patients were screened on the Illumina Human Cyto-12 chip. We will present results of the first analysis of the Dutch cohorts that shows that genetic risk factors for ACPA positive RA indeed differ from ACPA negative RA.

P09.106
A canine model for Hashimoto´s disease

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Hashimoto's thyroiditis, an autoimmune disease resulting in hypothyroidism, affects millions of people around the world. Even though some familiar cases have been genetically explained, the complete genetic basis of the disease remains largely unknown.
The dog, with its unique genetic structure formed by domestication and recent breed creation, has been proven to be an excellent model for mapping genes. Some breeds, such as the giant schnauzer, show an extraordinary high prevalence of naturally occurring hypothyroidism. We have carefully characterized the canine phenotype and believe there is a strong comparative value because of similar etiology, clinical signs and disease progression.
By a candidate gene approach we have found a strong association to DLA-DRB1*01201/DQA1*00101/DQB1*00201 (corresponding to HLA) in the Giant schnauzer breed (odds ratio of 6.5). We have also performed genome-wide association in the same breed, using the Illumina 174 K canine SNP array for 69 cases and 49 controls. Among the identified candidate regions, several harbour genes with known immuno-regulatory function. Fine-mapping in multiple breeds has confirmed the associations and identified a shared haplotype.
Currently we are performing functional analyses and targeted resequencing of candidate regions and genes in both dogs and humans in order to identify risk factors. We hope that our knowledge will lead to better diagnosis and treatment for both dogs and humans.

P09.107
Gender and dietary factors modulate the effect of the R230C/ ABCA1 variant on HDL-cholesterol levels in Mexican individuals

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The non-synonymous R230C variant within the ATP-binding cassette transporter A1 gene (ABCA1) has been associated with lower high-density lipoprotein cholesterol (HDL-C) levels in several independent studies. The aim of this project was to analyze whether gender, body mass index and dietary factors modulate the effect of R230C on HDL-C levels. The study included 3630 adults (1173 men and 2457 women) from a nationwide survey conducted by the State's Employees Social Security and Social Services Institute in Mexico (ISSSTE). Genotyping was perfoemd using Taqman probes. The overall risk allele frequency was 9.2%, showing a north to south increasing gradient, consistent with the higher contribution of the Native American gene pool to the populations of Southern Mexico. As expected, the C variant showed a highly significant association with lower HDL-C levels (mean TT:45.5 mg/dL; CT:41.9 mg/dL; CC:39.9 mg/dL; $p=9.2x10^{-13}$). However, there were clear gender differences, as the effect of the C variant was greater and more significant in women (β= -3.6%; $p=2.2x10^{-11}$) than in men (β= -2.8%; $p=1.8x10^{-3}$). In male subjects, increased percentage of dietary carbohydrates was significantly associated with lower HDL-C levels, but this effect was not observed in males with CT or CC genotypes (p= $p=7.3x10^{-4}$). In contrast, in women higher percentage of dietary carbohydrates was significantly associated with lower HDL-C only in in CT or CC genotypes (p=0.027), particularly in premenopausal women ($p=1.3x10^{-9}$). In conclusion, gender, menopausal status and percentage of carbohydrate intake modulate the effect of the ABCA1-R230C variant on HDL-C levels.

P09.108
Allelic polymorphisms of thrombophilic genes in children with brain diseases from Russia.

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Inherited thrombophilia (IT) can be defined as a genetically determined predisposition to the development of thromboembolic complications. However this problem is not fully clarified. The aim of our study was estimate allelic polymorphisms of different genes involved in IT in children with acute disturbance of brain hemodynamics and neurodegenerative diseases, and to estimate the impact of IT on the risk of stroke in children. The frequencies of polymorphisms -455G>A of the *FBG* gene, 20210G>A of the *FII* gene, 1691G>A of the *FV* gene, 1565T>C of the *GPIIIa* gene, 4G>5G of the *PAI-1*gene and 677C>T of the *MTHFR* gene were investigated in 20 children with ischaemic stroke, 58 children with neurodegenerative diseases and 100 age-matched controls, using hybridization with oligonucleotide biochips.
The frequency of the *FV* Leiden mutation was significantly higher in children with stroke (p=0.0001) compared with the control group. The occurrence of polymorphisms *MTHFR* 677C>T with 4G/5G in *PAI-1* was higher in children with stroke (p=0.05) and children with neurodegenerative diseases (p=0.003) compared with the control group. Thus our data have shown that the *FV* Leiden mutation can be considered as an important risk factor for ischaemic stroke. A combination of the *MTHFR* 677C>T variant and 4G/5G in *PAI-1* increases the risk of ischemic stroke and neurodegenerative diseases. Screening for inherited mutations may be helpful in the prevention of thrombophilic disorders.

P09.109
HHEX is associated with T2D in a South Asian Population

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The numbers of individuals worldwide developing T2D has increased over the last 30 years, with specific ethnic populations being at a higher risk for developing this complex disease. South Asians are one such population, particularly within an obesogenic environment. A compounding factor for this increased risk is body fat distribution and South Asian (SA) individuals, they have a higher volume of subcutaneous and central visceral adipose tissue but relatively less muscle mass. This contributes to them developing insulin resistance and T2D at a lower BMI, compared to Caucasians. Several genes have

been identified as candidates for T2D through genome wide association scans, including HHEX. Our study was designed to ascertain whether variants in the HHEX locus of 663 SA women influenced the risk of developing T2D. Using the Sequenom iPLEX assay 11 SNPs spanning this region were genotyped. Logistic regression controlling for age and BMI revealed two SNPs significantly associated with development of T2D, rs2488073 (p=0.021, OR 0.181 (0.043 - 0.7710)) and rs4933236 (p=0.004, OR 0.268 (0.109 - 0.66300)). In both cases the minor alleles show a protective effect towards T2D indicating that the risk alleles are the major alleles. Thus we have found an association with SNPs in the HHEX gene region and T2D that is specific to the South Asian population.

P09.110
A new contribution of *PHOX2B* in the pathogenesis of isolated Hirschsprung disease

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Hirschsprung disease (HSCR) is a congenital malformation of the hindgut produced by a disruption in neural crest cell migration during embryonic development. HSCR has a complex genetic aetiology with several genes involved in its pathogenesis. *PHOX2B* plays a key function in the development of neural crest derivatives, which has made it an excellent candidate gene for human neurocristopathies. In fact several mutations at this locus cause some syndromic forms of HSCR, as Congenital Central Hypoventilation Syndrome (CCHS) or neuroblastoma (NB). In contrast, to date no mutations had been associated to isolated HSCR and only the common variant g.1364A>G seems to have a predisposing effect in a Chinese series of patients. We sought to determine if *PHOX2B* plays any role in the pathogenesis of isolated HSCR, by both a mutational screening in our series, and the evaluation of its polymorphisms/haplotypes as modifier factors. No association was detected for the common variants tested, including g.1364A>G, which suggests that its association to HSCR is restricted to Chinese population. Our most relevant finding was the identification of a de novo and novel deletion (c.393_411del18) in a HSCR patient without any feature of NB or CCHS. Absence of this variant in 150 normal controls and results of in silico and functional assays support its pathogenic effect related to HSCR. This is the first time that a *PHOX2B* mutation is detected in an isolated HSCR patient, which represents a new perspective of the PHOX2B role in the pathogenesis of the disease.

P09.111
Evidences of synergistic effect of *RET* common and rare variants in a series of Spanish Hirschsprung patients

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Hirschsprung disease (HSCR) is a congenital malformation of the hindgut produced by a disruption in the neural crest cells migration during embryonic development. This disorder results in an absence of intramural ganglion cells in the submucosal and myenteric plexuses producing a functional intestinal obstruction. HSCR presents an estimated incidence of 1/5000 live births with sex-dependent penetrance and male predominance of 4:1. *RET* is the major gene associated to HSCR with differential contributions of its rare and common, coding and noncoding mutations to the multifactorial nature of this pathology. In the present study, we have performed a comprehensive study of our HSCR series evaluating the involvement of both *RET* rare variants (RVs) and common variants (CVs) in the context of the disease. RVs were identified by dHPLC and direct sequencing whereas studies of CVs, including the main CV associated to HSCR, the enhancer variant of intron 1 (rs2435357), were performed by Taqman technology. Our results confirm the strongest association to HSCR for the enhancer variant, and demonstrate a significantly higher impact of it in male versus female patients. Integration of the *RET* RVs and CVs analysis showed that in 91.66% of cases with both kinds of mutational events, the enhancer allele is *in trans* with the allele bearing the *RET* RV. Thus, *RET* CVs and RVs seem to act in a synergistic way leading to HSCR phenotype. Furthermore, our results show the existence of a gender effect on both the transmission and distribution of rare coding and common HSCR causing mutations.

P09.112
Novel identification of *PROKR1* and *PROKR2* genes implicated in the pathogenesis of Hirschsprung´s disease

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The enteric nervous system (ENS) is entirely derived from migrating neural crest cells and its development strongly depends on specific molecular pathways. Disturbances appearing during theses processes have been demonstrated to affect the proper formation and/or function of the ENS, leading to severe intestinal dysfunctions such as Hirschsprung´s disease (HSCR). Recently, the prokineticin receptor 1 (PROKR1) pathway has been identified to work co-ordinately with the RET/GDNF pathway in the development of mice ENS. In this sense, human ENS progenitors were isolated and characterized from the ganglionic gut obtained from childrens diagnosed with and without HSCR, using full-thickness gut resection specimens or gut biopsy samples, respectively. The expression of both prokineticin receptors, PROKR1 and PROKR2, was then examined. In addition, we also performed a mutational analysis of *PROKR1, PROKR2* and their ligands, *PROK1* and *PROK2*, in a cohort of HSCR patients, evaluating them for the first time as susceptibility genes for the disease. Immunocytochemical analysis showed that the majority of the ENS progenitors isolated and grown like neurosphere were multipotent and neural crest cells in origin. Furthermore, we demonstrated that not only PROKR1, but also PROKR2 might mediate a complementary signalling to the RET/GFRα1/GDNF pathway supporting proliferation/survival and differentiation of precursor cells during ENS development. These results, together with the detection of sequence variants in *PROKR1, PROK1* and *PROKR2* genes associated to HSCR and usually in combination with *RET* or *GDNF* mutations, provide the first evidence to consider them as susceptibility genes for HSCR.

P09.113
HLA-B27 gene study in a paediatric cohort of spondyloarthropathies

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Background

The spondyloarthropathies (SpA) are a family of related disorders that includes ankylosing spondylitis (AS), reactive arthritis (ReA), psoriatic arthritis (PsA), spondyloarthropathy associated with a inflammatory bowel disease (IBD), undifferentiated spondyloarthropathy (USpA). The spondyloarthropaties are strongly associated with HLA-B27 gene.

Aims

The objective of our study was to observe the correlations between the positivity of HLA-B27 gene and the clinical, biological manifestations of the patients, the response to the treatment and the course of the disease.

Material and methods

We tested for HLA-B27 gene a group of 19 pediatric patients linked by common pathology, including inflammatory back pain and peripheral enthesitis. The evaluation of the patients included complete clinical assessment, laboratory studies, radiological findings and genetic study of HLA-B27.

Results
According to the European Spondyloarthropathy Study Group (ESSG) criteria, we established the diagnoses from the tabel below and the presence of HLA-B27 gene.
Six B27-positive patients from the total 13 children with SpA responded inadequate to non-steroidal anti-inflammatory drugs (NSAID), needing forward treatment (Sulfasalazine, Mesalazine, biologic agents), comparing with the B27-negative children, who had excellent response to NSAID. The course of the disease was more favorable in HLA-B27 negative children.
Discussions
HLA-B27 gene represents a diagnostic criteria in SpA, but could be used as a marker for the treatment response and disease outcome.

HLA-B27 presence in SpA			
	HLA-B27 positive	HLA-B27 negative	Total cases
USpA or Enthesitis-related arthritis	4	1	5
IBD associated SpA	2	1	3
ReA	2	3	5
Patients with cronic back pain, but without criteria for SpA	1	5	6

P09.114
Characterisation of HLA-DQB1 expression demonstrates highly variable expression regulated by SNPs associated with Leprosy susceptibility

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The HLA-DQ1 heterodimer is composed of DQA1 and DQB1 proteins that together form an antigen presentation molecule expressed on most antigen presenting cells. DQA and DQB alleles are strongly associated with susceptibility to multiple diseases, most notably autoimmune conditions including coeliac disease and rheumatoid arthritis. Much of the genetic variation between DQ alleles leads to coding changes resultant in varying abilities to bind and present certain peptides. In addition to structural variation between DQA1 and DQB1 alleles, it has become increasingly apparent that alleles vary in terms of quantitative expression. The highly polymorphic nature of these genes can confound microarray based expression quantitative trait (eQTL) analysis by interference with probe hybridization. With this in mind we performed eQTL analysis of these genes in peripheral blood mononuclear cells from 288 healthy Caucasians using quantitative PCR. Subjects were genotyped at 731,442 markers (Illumina HumanOmniExpress) with subsequent imputation analysis. We identified a haplotype associated with a 5-fold increase in baseline HLA-DQB1 expression (rs9273440, p 3.2*10-66). Whilst this haplotype does not appear to be associated with autoimmune disease, it includes risk alleles for the mycobacterial disease Leprosy. Further analysis of Leprosy associated SNPs demonstrate that in tandem with increased susceptibility noted with increased HLA-DQB1 expression, SNPs associated with reduced HLA-DQB1 expression are protective against Leprosy. These associations were not seen with HLA-DQA1 or HLA-DRB1 expression. This analysis advances our understanding of the strong genetic association of Leprosy with the MHC class II region and provides functional insights into mechanisms of Leprosy susceptibility.

P09.115
Correlation of MTHFR C677T polymorphism with plasma homocysteine concentration in Armenian patients with ishemic stroke and acute myocardial infarction

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In view of the controversy of the role of MTHFR C677T mutation in plasma homocysteine (Hcy) level elevation in patients with cardiovascular diseases and paucity of studies from Armenia, this study has been undertaken. The overall aim was to evaluate MTHFR C677T gene polymorphism in ischemic stroke (IS) and acute myocardial infarction (AMI) patients and correlate these with Hcy level. In a study group of 54 patients (43% male) with confirmed IS, 38 patients with AMI (87% male) and 56 healthy volunteers (50% male), MTHFR polymorphism was detected by SNP-extention assay and determined by ELISA (Prontodiagnostic), and total plasma Hcy was measured by ELISA (Axis-Shield). Significant increase of Hcy was detected in patients with IS (19,18+/-1,49 micromol/L) and AMI (15,59+/-1,76 micromol/L) compared to controls (10,01+/-0,86 micromol/L). The OD for IS in subjects with hyperhomocysteinemia was 16,7 (95%CI=6,05-45,6, p=0.001) and for AMI was 8,3 (95%CI=2,9-24.1, p=0.01). Meantime, the elevation of Hcy level above 15 micromol/L was observed in **56% of cases with IS and in 42% with AMI, while only in 5% of controls.** The plasma Hcy in patients with IS and AMI with MTHFR CC, CT and TT genotypes were 16,6±1,7, 21,20±1,7, 30,12±9,6 micromol/L and 13,11+/-1,24, 18,25+/-3,19, 16,27+/-2,57 micromol/L, respectively. Thus, we showed association of MTHFR polymorphysm in IS and AMI patients with high frequency of elevated homocysteine level in comparison with control group. However, since homocysteine level in latter don't depend on MTHFR polymorphism, e**xponentiation of homocysteine level by other risk factors are quite possible.**

P09.116
RET Mediated Gene Expression Profiling of ENS Precursors

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Signaling of the RET-GDNF receptor triggers many important signal transduction routes crucial for the migration, proliferation, differentiation and survival of the neural crest stem cells (NCSCs), cells that are responsible for the formation of the enteric nervous system (ENS). To identify pathways and genes triggered by RET, we performed gene expression profiling using Microarray Affymetrix GEneChip Mouse Genome 430 2.0 platform on GDNF stimulated and non-stimulated NCSCs. For this we isolated YFP labeled-NCSCs out of embryonic mouse guts E14.5 from Wnt1-Cre/LoxP/R26-YFP transgenic mice by FACs sorting and short term cultured in media with or without GDNF. To identify NCSCs specific genes, we compare the microarray data of NCSCs and embryonic gut E14.5. The data were analyzed by Gene Set Enrichment Analysis (GSEA) and single-gene analysis methods. Pathways that were up-regulated during GDNF stimulation were pathways important for Lipid metabolism, ATP and Steroid synthesis. Several important signaling pathways for ENS and embryonic development were found to be significantly down-regulated upon GDNF stimulation such as the Notch, TGFβ/Smad and Wnt signaling pathways. Genes involved in the cell cycle arrest and apoptosis were down-regulated, pointing toward a model in which NCSC proliferate and survival during ENS development. By single-gene analysis, we identified 429 genes that were differentially expressed between NCSCs treated with GDNF and untreated cells (Bonferoni, $p < 0.05$). In summary, here we provide a profile of pathways and genes that are expressed in ENS precursors and regulated by the RET signaling. These data might helpful in identifying candidate HSCR genes.

P09.117
The role of functional HTR1B variants in alcohol dependence: evidences from haplotype analysis

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Alcohol dependence, a common and clinically heterogeneous disease, is frequently comorbid with many other mental illnesses and influenced by genetic and environmental factors. Animal and human studies have suggested that the serotonergic system plays an important role in alcohol consumption and abuse, mainly due to the serotonin receptor 1B (5-HT1B) function in the mesolimbic reward pathway. Association studies between the HTR1B gene variants and alcoholism have found significant results. There is also evidence for a complex balancing regulation of this gene by two functional variants in its promoter region (T-261G and A-161T), which are in linkage disequilibrium. The aim of this study is to investigate the role of the most relevant variants of the HTR1B gene (T-261G, A-161T, G861C and A1997G) in the susceptibility to alcohol dependence. The sample comprised 136 Brazilian alcoholics of European descendent and 237 controls. The results suggest an association between a functional variant of the gene (T-261G) and alcohol dependence (p=0.001). The haplotype analysis,

comprising all studied HTR1B polymorphisms, has shown a different distribution pattern between patients and controls (p<0.0001), which is consistent with the role of the two functional variants of the promoter region. In conclusion, our findings point to an association between functional variants in the promoter region of the HTR1B gene and alcohol dependence, supporting previous neurobiological evidences of the involvement of HTR1B variations in alcohol-related phenotypes.

P09.118
Human *SA* gene polymorphisms are associated with hypertension in the Chinese population: the SAPPHIRe study

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Previous studies found significant association of hypertension and hypertension-related phenotypes with genetic variation in the *SAH* (Spontaneously hypertensive rat-clone A - Hypertension-associated) gene. This study aimed to examine whether common *SAH* genetic variations are associated with hypertension in the Chinese population. We genotyped three single-nucleotide polymorphisms (rs55810929, rs11647477, rs5716) of the *SAH* gene in 894 subjects of Chinese origin from the Stanford Asia-Pacific Program for Hypertension and Insulin Resistance (SAPPHIRe) family study. All three SNPs in the *SAH* gene were significantly associated with risk of hypertension in the SAPPHIRe cohort (p= 0.026 for rs55810929, p=0.007 for rs11647477, p=0.009 for rs5716). The subjects carrying with the DI or II genotypes of the rs55810929 SNP was associated with hypertriglycerides (>=150 mg/dL) as compared with the DD genotype (p = 0.039). Moreover, both rs11647477 and rs5716 SNPs were found to be associated with excess waist circumference (p=0.025 and p=0.015 from chi-square for trend test). Furthermore, four haplotypes were constructed based on the rs11647477 and rs5716 polymorphisms. Subjects carried haplotype AC was associated with increased risk of hypertension (p=0.01 from HBAT permutation test); while haplotype GG was conferred protective effect of hypertension (p=0.006 from HBAT permutation test). In conclusion, our results suggest the polymorphisms of the SAH gene are significantly associated with hypertension, elevated levels of tryglycerides, and excess waist circumference. Our study validated the association between SAH gene polymorphisms and hypertension in the Chinese population.

P09.119
Homocysteine level in Ukrainian patients regarding to polymorphic variants of genes, involved in folate metabolism

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Hyperhomocysteinemia is generally established etiological factor for arterial and vein thrombosis, and several polymorphic variant's of genes, involved in homocysteine remethylation (C677T and A1298C of MTHFR gene, A2756G of MTR gene and A66G of MTRR gene), are recommended for genetic testing in cases of increased risk of thrombophilia. Among 84 Ukrainian patients with high risk of thrombophilia complications (42 with preeclampsia and 42 with coronary artery disease) the polymorphic variants of genes, involved in folate metabolism, were analyzed regarding to plasma homocysteine level. The homocysteine level was varied between 4,48 to 31,97 mkMol/l in examined cases and there was no significant difference in groups of patients with preeclampsia and coronary artery disease: 9,08±3,06 and 9,88±2,12 mkMol/l respectively (P>0,05). 677TT genotype of MTHFR gene was marked for highest plasma homocysteine concentration and heterozygoes 677CT genotype was the following (11,54±2,76 mkMol/l and 9,92+0,53 mkMol/l respectively, P>0,05), whereas in 677CC carriers homocysteine level was significantly lower (8,57±0,36, P<0,05). No significant differences in homocysteine plasma blood concentration were established in different combinations of C677T and A1298C gaplotypes of MTHFR gene, in cases of AA, AG, GG genotypes of A2756G polymorphic variant of MTR gene (9,45±2,97, 9,54±1,80 and 9,23±2,55, mkMol/l respectively, P>0,05), and also in cases of AA, AG, GG genotypes of A66G polymorphic variant of MTRR gene (9,04±1,90, 9,25±0,47 and 9,81±3,26 mkMol/l, correspondingly P>0,05). In conclusion, significantly increased plasma homocysteine concentration was found in carriers of 677TT and 677CT genotypes of MTHFR gene among Ukrainian patients with preeclampsia and coronary artery disease.

P09.120
Association of prostaglandins receptors polymorphisms with cutaneous hypersensitivity reactions induced by non steroidal anti-inflammatory drugs.

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RATIONALE: NSAIDs are actually the compounds most frequently involved in hypersensitivity drug reactions, and there are at least three different mechanisms involved: IgE- mediated, and T cell-mediated (immunological specific mechanisms) and cross-intolerance (CI) (immunological non-specific). In the latter group skin is the main affected organ. Associations between CI and different SNPs in arachidonic acid pathway or prostaglandins receptors (PGsR)-related genes had been described in patients with respiratory symptoms or chronic urticaria, but up today the number of cases is rather limited and selected populations are heterogeneous. In this study, we analysed the association between CI and several SNPs in PGsR in a large group of patients with acute urticaria/angioedema (AUA), defined as skin episode in absence of airways involvement or coexistent chronic urticaria.

METHODS: The population was obtained from several Allergy Services integrated into the Spanish network RIRAAF. Cases included had to have at least two episodes of CI with cutaneous symptoms after the intake of two or more NSAIDs from different chemical groups. We studied several SNPs in *PTGER1*, *PTGER2*, *PTGER3*, *PTGER4*, *PTGDR*, and *PTGFR* genes by using the TaqMan®OpenArray™ System.

RESULTS: We included 249 subjects with AUA, and 247 age- and sex-matched healthy controls. We found statistically significant associations between AUA and *PTGDR* rs8004654, *PTGER1* rs3810255, and *PTGER2* rs1254598.

CONCLUSIONS: Our results suggest the importance of genetic variants in PGsR in NSAIDs-induced AUA. Nevertheless further studies are required to analyze the role of other variations in PGsR and related genes in this clinical entity.

P09.121
Combine effect of Factor V Leiden, MTHFR and Angiotensin converting enzyme (insertion/deletion) gene mutations in hypertensive adult individuals: a population-based study from Sivas and Canakkale, Turkey

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Hypertension is one of the leading causes of mortality and morbidity in the world which influenced by environmental and genetic factors. The MTHFR and Angiotensin converting enzymes (ACE) are possible candidate genes that may influence both body fatness and blood pressure. The purpose of this study was to examine the carriage of gene combinations of the ACE (I/D), MTHFR 677T, 1298C and lipid profiles in patients with essential hypertension (EH) in Turkey.

In a total of150 adult individuals (50 hypertensive, 50 first degree

relatives and 50 healthy control) from Sivas/Turkey with the same age, gender assessed for body composition, lipid profiles, resting blood pressure and gene profiles. Additionally, 149 individuals (99 hypertensive, 50 controls) from Canakkale/Turkey had been investigated for ACE I/D polymorphism. Pheripheric blood samples were genotyped using stripassay reverse hybridisation multiplex PCR tests for target genes.
Heterozygote mutation in FV Leiden was found to be higher in the hypertensive and first degree relatives when compared to the control group($p<0.05$). Homozygous DD allels of ACE gene were also higher than the ACE I/D and control groups ($p<0.05$). The high rates of cholesterol, LDL and low rates of HDL were found in patients with EH when compared with the control.
Current results show that ACE with DD allels, mutated allels of FV Leiden and MTHFR genes were significantly different between genotypes and have a combine effect on EH in Turkish population. Further studies are needed to investigate the genetics of fatness, EH and BP phenotypes in the current adult population.

P09.122
Polymorphism Ile105Val of the *GSTP1* gene is a predictor for ischemic stroke in patients with essential hypertension in Russian population

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The present study was designed to test whether two common polymorphisms Ile105Val and Ala114Val of glutathione S-transferase (*GSTP1*) gene are associated with risk of ischemic stroke (IS) in patients with essential hypertension (EH) in population of Central Russia. A total 607 unrelated Russian subjects, including 300 EH patients after MRI-diagnosed cerebral ischemic stroke (EH-IS group) and 307 EH patients having no cerebrovascular events (EH-group) were recruited in the Kursk regional hospital for a period 2008-2010. The polymorphisms were genotyped through PCR-RFLP assays. The frequency of allele 105Val of *GSTP1* gene was significantly higher in EH-IS group (0.418) versus EH one (0.336), odds ration 1.42 95%CI 1.13-1.80 p=0.004. The frequency of genotype 105ValVal of *GSTP1* gene was 19.0% and 9.8% in EH-IS and EH groups, respectively. The genotype 105ValVal of *GSTP1* gene was associated with increased risk of IS in EH patients (odds ration 2.15 95%CI 1.34-3.44, p=0.002). No difference in allele and genotype frequencies between the groups was found for the other genes. This is the first study reporting on the association of polymorphism Ile105Val of the *GSTP1* and ischemic stroke in hypertensive patients. The study was supported by the Federal Targeted Programme “Scientific and Scientific-Pedagogical Personnel of the Innovative Russia in 2009-2013”.

P09.123
Gene-environment interactions as explanation for genetic non-replication in the etiology of hypospadias

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Hypospadias is a common congenital malformation of the male external genitalia. In previous studies, hypospadias was associated with single nucleotide polymorphisms (SNPs) in *SRD5A2*, *ESR1*, *ESR2*, and *ATF3*. However, we were unable to replicate these associations in a Dutch study. Therefore, we examined whether this lack of replication could be due to dissimilar environmental exposures, by exploring whether the associations differed when mothers were or were not exposed to exogenous estrogens, suffering from placental insufficiency, or having high estradiol levels. For *ATF3*, we also included occurrence of an infection and/or inflammation and smoking during pregnancy. We genotyped 712 hypospadias cases and their parents, obtained environmental data from postal questionnaires, and tested the presence of gene-environment interactions using the log-linear approach. Gene-environment interactions were identified between rs523349 in *SRD5A2* and maternal estrogen exposure and between rs11119982 in *ATF3* and the occurrence of an infection and/or inflammation. The SNP in *SRD5A2* only increased the risk of hypospadias when the mother was exposed to exogenous estrogens. This could explain why we were unable to confirm the associations found in a Chinese and a Swedish study. Possibly, Chinese and Swedish women experience higher exposures to phytoestrogens than Dutch mothers, because they consume more soy products, rye bread, and berries. The previously reported decreased risk for rs11119982 could not be confirmed, as we found an increased risk of hypospadias when the mother had an infection and/or inflammation and no effect when the mother did not. In conclusion, environmental factors can explain genetic non-replication between studies.

P09.124
High-resolution melting curve analysis for high-throughput genotyping of NOD2/CARD15 and IL23R SNPs and their distribution in Slovenian inflammatory bowel diseases patients

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Inflammatory bowel diseases (IBD) are usually classified into Crohn’s disease (CD) and ulcerative colitis (UC). Previous studies in Caucasians have shown that the most potent IBD associated single nucleotide polymorphisms (SNPs) reside in NOD2/CARD15 and IL23R genes (rs2066844, rs2066845, rs2066847 and rs7517847). High-resolution melting analysis (HRMA) was previously reported as a simple, inexpensive, accurate and sensitive method for genotyping and/or scanning of rare polymorphisms. Therefore, qPCR-HRMA was used for genotyping of NOD2/CARD15 and IL23R polymorphisms in 588 Slovenian IBD patients and 256 healthy controls. These results were compared with genotyping results obtained by PCR-RFLP, which were used as a reference method. The optimization of an HRM experiment required careful design and adjustment of main parameters, such as primer concentration, MgCl2 concentration, probe design and template DNA concentration and different HRMA approaches were tested develop reliable and low-cost SNP genotyping assays. Direct HRMA was the fastest and cheapest approach for rs2066847, rs2066844 and 7517847 polymorphisms, yet for rs2066845 polymorphism sufficient reliability was achieved after introduction of unlabeled probe. Association analysis revealed significant associations of rs2066847 ($p = 0.001$, OR = 3.011, CI95% = 1.494-6.071) and rs2066845 ($p = 2.62 \times 10^{-4}$, OR = 14.117, CI95% = 1.884-105.799) polymorphisms with CD patients. The rs7517847 polymorphism was significantly associated with both, CD ($p<0.001$, OR=0.588 CI95% = 1.346-4.797) and UC patients ($p = 0.035$, OR=1.599 CI95% = 1.048-2.439). Applied HRMA assays may contribute to the development of genetic profiles for risk prediction in IBD and for differential diagnosis of CD vs. UC.

P09.125
The IL10 promoter polymorphisms is associated with both CD and UC in Tuscany population

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Pathological evidence supports a potential role of the pro-and anti-inflammatory cytokine network in the pathogenesis of IBD. In this study, we evaluated the effect of the anti-inflammatory interleukin-10 (IL10) gene on Ulcerative Colitis and Crohn’s Diseasae. Two SNPs in the IL10 promoter, -1082G>A (rs1800896) and -819C>T (rs1800871), were determined in 553 patients (315 UC and 238 CD) and 367 healthy controls from Tuscany (Italy) by HRMA. Statistical analysis was performed using EpiInfo software to assess the risk and using Power Marker software for haplotype frequencies. The -1082G>A SNP followed the HW equilibrium in both UC and CD patients and controls. The HW equilibrium for the -819C>T SNP was respected only in the control group and not in the UC and CD cases. This may be further evidence of the association of the locus with the disease. The IL10-819T allele was weakly associated in UC patients and more significantly with CD patients, compared to controls. The frequency of the IL10-1082A allele was significantly higher both in UC and in CD patients than in controls. In addition, we observed an higher frequency

of the IL10 A/T haplotype in both UC and CD patients, compared to controls. Moreover, we found that -1082G>A SNP was in low LD with -819C>T both in UC and CD cases and controls. In conclusion, the -1082G>A and -819C>T SNPs in the IL10 promoter were significantly associated with risk of UC and CD in a population of Tuscany, suggesting their role in IBD susceptibility at least in this population.

P09.126
Interleukin-1 β and Interleukin-1 Receptor Antagonist Gene polymorphisms in children not responding to Hepatitis B vaccination

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Introduction: Hepatitis B virus (HBV) infection is a major public health issue throughout the world and vaccination of those at risk is the main method of containment. 5-10% of children are not responding to hepatitis B vaccines and probably are not adequately protected against the (HBV) infection. We aimed to investigate the genetic polymorphisms of interleukin-1B (IL-1B) and interleukin-1 receptor antagonist gene (IL-1RN) in children not responding to hepatitis B vaccination, and to determine whether vaccine efficacy is influenced by these gene polymorphisms.

Material and Method: A total of 200 children whose anti-HBs antibody levels lower than 10 IU/L after vaccination against hepatitis B according to a standardized schedule were included in this study as non-responder group. One hundred healthy children who had anti-HBs antibody levels higher than 10 IU/L after vaccination against hepatitis B were included as responder group. IL-1B -511(C/T) and IL-1RN VNTR gene polymorphism were determined using PCR-RLFP and PCR-agarose gel based techniques, respectively.

Results: Frequencies of the genotypes and alleles of IL-1B -511(C/T) polymorphism were similar in both group. Genotype frequencies of IL-1RN VNTR polymorphism were not significantly different between responders and non-responders group. The frequency of allele 2 of IL-1RN VNTR polymorphism was higher in non-responders than responders group (respectively; r= 0,17, r=0,09, p=0,029). The allele 1 and allele 3 frequencies were not different in both group (respectively; p=0,86, p=0,48).

Conclusion: Our results suggest that, allele 2 of IL-1RN gene VNTR polymorphism might be associated with not responding to hepatitis B vaccination.

P09.127
Analysis of copy number variants (CNV) distribution in high scoring impulsive-disinhibited subjects

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Impulsivity/disinhibition trait has been defined as the predisposition to respond to internal or external stimuli without regard to the potentially negative consequences to the individual or to others. The genetic of personality traits has been evaluated in relation to microsatellites and SNPs. Nevertheless, the contribution of CNV is not known. CNVs produces an increase or decrease in the number of copies of one or several genes, which can affect their expression and regulation. We hypothesize that CNV may be part of the genetic component of the impulsivity/disinhibition trait. To test this hypothesis we have performed a comparative genome hybridization (CGH) analysis with the Agilent 2X400K CGH array in a group of subjects whose scores in impulsivity/disinhibition questionnaires were the highest. DNA from each high scoring subject was compared with a pool of DNA obtained from low scoring controls. We used the ADAM2 statistical algorithm to identify CNVs and CNV regions (CNVR) with statistical threshold of 6 and a minimum of 4 probes by using Agilent Genomic Workbench. With these stringent conditions we detected 3310 CNVRs in a subset of 16 subjects that passed array QC. CNVR were distributed according to chromosome size. A total of 4430 GO annotated genes were mapped to 2825 (86%) CNVRs. An overrepresentation of GO:0005488 binding category genes was observed (3200 genes, 72%). In addition, genes included in the GO categories synapse (120 genes, 2,7%) and reproduction (315 genes, 7.1%) were detected with strong statistically significance. The in depth characterization of results is in progress.

P09.128
eQTL analysis of disease associated regions in Slovenian inflammatory bowel disease patients

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In many disease associated regions of complex diseases such as inflammatory bowel diseases (IBD) SNPs and haplotypes most significantly associated with disease are located in non-coding regions. The mechanism by which these SNPs contribute to the disease pathogenesis and which genes and proteins they affect is not yet clear. Using method of linking SNP genotype to the level of gene expression (expression quantitative trait loci - eQTL) we wanted to explore the feasibility of eQTL analysis for linking non-coding disease associated SNPs to genes they regulate. Initially we analyzed locus from which non-coding SNP rs3087243 (CTLA4_CT60) showed functional relevance and has been associated with the disease. We further applied our approach to IBD5 region where SNPs are in high linkage disequilibrium and the main contributor from this region is still unknown. We measured expression of genes from selected regions in peripheral blood lymphocytes (PBL) and colon tissue biopsies of 632 Slovenian IBD patients and in PBL of 312 controls and genotyped all patients and controls for selected SNPs. We showed that non-coding SNP rs3087243 affect expression of soluble isoform of CTLA4 gene. Our eQTL analysis of IBD5 region suggested SLC22A5 as a main gene in that region contributing to the IBD pathogenesis where decreased expression of SLC22A5 gene for disease susceptible genotype of SNPs rs1050152 and rs2631372 was detected. Our results suggest genotype/gene expression studies are good approach in linking most significantly disease associated SNPs in non-coding regions with genes they regulate.

P09.129
Genome-wide screen for context-dependant associations reveals an interaction between a locus at 4p15 and waist-to-hip ratio on total cholesterol

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A large-scale (>100,000 individuals) genome-wide association study recently described numerous loci affecting lipid levels. These common variants explain ~25% of the heritability of these phenotypes. A number of hypotheses have been proposed speculating on the sources of such “missing heritability”, including context-dependant genetic effects due to interactions. In the current analyses, the role of interactions influencing lipids was explored in meta-analysis of genome-wide data from 19 population-based cohorts (n>32,000). Studied lipids were total (TC), high-density lipoprotein, and low-density lipoprotein cholesterol and triglycerides. The interacting variables, chosen for their prior associations with lipids, were alcohol consumption, smoking, sex, waist-to-hip ratio (WHR), and body-mass index. Eight in silico replication cohorts (n>14,000) were available. Interactions were tested using linear regression models including both main effects and a SNP*context interaction term. After meta-analyzing the discovery and replication samples, rs6448771, near the protocadherin 7 gene (PCDH7), showed genome-wide significant interaction with WHR on TC (P-value=9.08e-9). A 10% increase in WHR elevated TC by 0.14 mmol/L (95% CI:0.10-0.18) for the AA genotype, 0.20 mmol/L (CI:0.16-0.24) for AG, and 0.22 mmol/L (CI:0.15-0.29) for GG. The SNP alone did not affect either WHR (P-value=0.46) or TC (P-value=0.51). No other genome-wide associations were observed. Inclusion of suggestive SNP*context interactions in polygenic models marginally improved

the proportion of explained variance. In conclusion, rs6448771 represents the first SNP identified by a genome-wide search for SNP* risk-factor interactions affecting lipids. It is unlikely that large effect-size interactions between common variants and the tested interacting contexts explain a high proportion of "missing heritability".

P09.130

Using canine models in the study of human disease: Genome-wide association mapping identifies a major locus affecting intervertebral disc calcification in Dachshund

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In humans, genetic studies of intervertebral disc degeneration have mainly focused on candidate gene studies, but the etiology and pathogenesis of disc degeneration is still poorly understood. Studies of the equivalent disease in dogs offer a relevant animal model to investigate the genetics underlying the phenotype. The predisposition to intervertebral disc disease in Dachshund is caused by an early degenerative process resulting in disc calcification. In Dachshund the number of calcified discs at two years of age determined by a radiographic evaluation is a good indicator of the severity of disc degeneration and thus serves as a measure for the risk of developing intervertebral disc herniation. Since the assessment of disc degeneration in humans often is performed by magnetic resonance imaging where it is difficult to evaluate disc calcifications, it is not possible to make a direct comparison between the etiology of the disease in the two species. However, elucidation of the biological processes involved in disc degeneration in dogs will undoubtedly also shed light on the pathways relevant for the human condition. Based on stringent radiographic examinations 48 affected dogs with ≥6 disc calcifications or surgically treated for disc herniation and 46 unaffected dogs with 0-1 disc calcifications were identified. Genome-wide association using the Illumina CanineHD BeadChip identified a locus on chromosome 12 from 36.8Mb-38.6Mb with 36 markers reaching genome-wide significance with p-values of $1.0x10^{-5}$-0.026 (p-values corrected for multiple testing). These results suggest that a major locus affecting the development of intervertebral disc calcification is present on chromosome 12.

P09.131

Factor XIII V34L polymorphism and their association with hematoma growth in patients with acute intracerebral hemorrhage

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We tested the hypothesis that the FXIIIV34L polymorphism is associated with hematoma growth (HG) in patients with acute intracerebral haemorrhage (ICH).

Methods: We studied patients with spontaneous ICH within the first 6 hours after the onset of symptoms. HG was defined as an increase >33% in the volume of hematoma on the computed tomography (CT) obtained 24 to 72 hours after the onset of symptoms in comparison with the CT at admission. We used *Chi-Squared* test to compare frequencies and the risk of HG was assessed as an odds ratio (OR) with a 95 % CI using logistic regression (adjustments for: age, sex, volume haemorrhage at baseline, onset to the first CT, blood glucose, blood pressure, Glasgow score). The FXIIIV34L, Factor V Leiden and *F2G20210A* were genotyped.

Results: We included 90 patients (mean age 71± 10.8y.), 61% were men. HG was observed in 35 (39%) of the patients. The frequency of HG was higher among patients who carried the L34 allele than patients with the V34 allele (51.6% vs 31.6% p=0.072). If patient who carried Factor V Leiden or *F2G20210A* were not included, the difference was significant (53.3% vs 29.6 %, p= 0.039). Carriers of L34allele showed a higher risk of HG (OR= 3.96; 95 % CI:1.4-11.2). Moreover, we found that every year of age increased 5% the risk of HG (OR= 1.05).

Conclusion: The L34 allele increases almost 4 times the risk of developing HG in patients with acute intracerebral haemorrhage.

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P09.132**

Intracranial Aneurysm risk locus 5q23.2 is associated to elevated systolic blood pressure in the Finns: A meta-analysis

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Background and Purpose - Previous GWA studies have identified 5 loci with strong association (posterior probability of association -PPA>0.5) and further 14 loci with suggestive association (0.1<PPA<0.5) to intracranial aneurysm (IA). However, the pathomechanism by which these loci increase the risk of IA formation remains mostly unknown. Hypertension is one of the strongest traditional risk factors of IA known to date. We hypothesized that some of the loci with strong or suggestive association to IA convey their effect through elevating blood pressure. We further hypothesized that this effect is more likely detectable in a genetically more homogenous population.

Methods - We performed a meta-analysis of four previously described Finnish cohorts consisting of 12,152 individuals. We used a two tier approach; one discovery cohort and three replication cohorts.

Results - We found that the suggestive IA locus at 5q23.2 in PRDM6 is strongly associated with systolic blood pressure (SBP) (p= 0.00016). The risk allele of IA was associated to higher SBP. PRDM6 encodes a protein playing a key role in vascular smooth muscle cell homeostasis. We hypothesize that common variants in PRDM6 may contribute to altered vascular wall structure hence increasing SBP, and predispose to IA.

Conclusions - To our knowledge, this is the first study unambiguously demonstrating replication of a complex disease locus (IA), with its common risk factor (SBP). Further validation is needed to show the significance of this association in other populations.

P09.133

TNF and CCR5 gene polymorphisms are associated with response to anti-TNF treatment in patients with juvenile idiopathic arthritis.

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Juvenile idiopathic arthritis (JIA) is a group of chronic arthropathies in children, caused by aberrant immune reactions, with predominance of Th1- over Th2-driven responses. It has been shown that Th1-chemokine receptor CCR5 is important in the recruitment of T-helper cells of children with JIA to the synovium, where they secrete pro-inflammatory cytokines and cause the inflammation. TNF-alpha is one of the major pro-inflammatory cytokines and it plays a key role in pathogenesis of JIA. Anti-TNF therapy has proved to be highly successful in controlling inflammation and is now widely used to

treat JIA. However, large percentage of patients is refractory to anti-TNF treatment. We investigated contribution of TNF-alpha -308 G/A polymorphism (up-regulation of TNF-alpha production) and Th1-chemokine receptor CCR5-delta32 polymorphism (non-functional receptor) on response to infliximab (a monoclonal antibody against TNF-alpha) in patients with JIA. We genotyped the two polymorphisms in 75 Russian patients with JIA and found that TNFA G allele frequency was significantly lower in infliximab responders versus non-responders (83,3%; 96,7%, respectively, p=0,013). The proportion of minor A allele was significantly higher in responders vs. non-responders (33,3% and 6,7%, respectively, p=0,008). Frequency of CCR5-delta32 was significantly lower in infliximab responders (1,4% versus 10,0% in the group of refractory patients; p=0,028). Our data might be useful for determination of management strategy for children with JIA. This work was supported by Russian Foundation for Basic Research, grant No. 09-04-13849.

P09.134**

Two novel genetic factors for CAKUT origin: results from the AGORA project

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Congenital anomalies of the kidney and urinary tract (CAKUT) occur frequently in man and comprise the most common cause of end-stage renal disease in children. Structural disorders belonging to the spectrum of these anomalies include renal agenesis, multicystic kidney dysplasia, ureteropelvic junction obstruction, duplex collecting system, and vesico-ureteral reflux. Not much is known about the origin of CAKUT. Alterations in genes expressed during nephrogenesis are considered to be important, with the final phenotypic outcome depending on additional modifying genetic and environmental factors. The aim of this study is to identify new genetic factors involved in CAKUT aetiology. From the AGORA biobank of the Radboud University Nijmegen Medical Centre over 700 well-documented CAKUT case-parent triads and 10 families with multiple affected members were recruited. Mutation analysis of two novel CAKUT candidate genes was performed and revealed interesting genetic variants that were functionally characterized *in vitro*. Genome-wide exome sequencing in CAKUT families identified variants possibly involved in CAKUT aetiology. In addition, linkage analysis of a large CAKUT family demonstrated suggestive linkage for a locus on chromosome 4. The identification of new genetic factors for CAKUT contributes to the understanding of the pathogenesis and the design of genetic diagnostic screening tests, facilitating early detection and recurrence risk estimations for CAKUT.

P09.135

Common genetic variants associated with kidney function and blood pressure are not associated with kidney volume in early life. The Generation R Study

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It has been postulated that smaller kidneys with reduced number of nephrons lead to impaired kidney function and risk for hypertension and chronic kidney disease. These associations may be explained by common genetic variants that cause lower nephron endowment and smaller kidneys and subsequent increased blood pressure or impaired kidney function. Therefore, the aim of our study was to assess the associations of common genetic variants which have previously shown to be related to adult kidney function and disease (30 common variants, P-value < 5.0*10-8), and blood pressure (23 common variants, P-value < 1.0*10-7), with kidney volume at the age of 6 years. This study was embedded in a population based prospective cohort study among 1233 children aged 6 years. Genotyping was done using the Illumina Human 610 Quad Arrays. If needed, imputation was performed using MACH.

Two of the common variants (in/near STC1, C18orf1) reached the nominal significance threshold (P<0.05) and showed the expected direction of the effect (i.e. lower kidney volume for a SNP previously associated to lower glomerular filtration rate in adults). However, when taking into account multiple testing, none of the common variants were associated with kidney volume at the age of 6 years.

Our results suggest that the underlying mechanisms in which common variants affect kidney function and blood pressure do not include altered kidney growth and development in early life.

P09.136

Role of KIR3D receptor functional variants in ankylosing spondylitis

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Some contradictory results on association of activating and inhibitory alleles of killer immunoglobulin-like receptor KIR3D gene with ankylosing spondylitis (AS) were reported. Only one of the studies analyzed the association in the context of functionality of polymorphic KIR3DL1. In our study we analyzed both the frequencies of alleles encoding inhibitory and activating KIR3D receptors and alleles encoding functional (KIR3DL1*F) and non-functional (KIR3DL1*004) inhibitory receptors in two Russian Caucasian cohorts which include 83 AS patients and 107 HLA-B27-positive healthy donors. Presence of low expressed KIR3DL1*005 and KIR3DL1*007 alleles was also detected.

The frequency of KIR3DS1 allele was found to be higher (30.7% vs 18.7%, $p < 0.01$, OR = 1.93) and the frequency of KIR3DL1 allele was found to be lower (69.3% vs 81.3%, $p < 0.01$, OR = 0.52) in the AS cohort. Also it was found that the KIR3DL1*004 allele frequency are near equal in both cohorts, and the decrease of KIR3DL1 allele frequency in the AS cohort is due to the KIR3DL1*F but not KIR3DL1*004 frequencies. Likewise the frequencies of genotypes carrying KIR3DL1*004 was found to be near equal in both cohorts. 9 from 17 KIR3DL1*F/KIR3DL1*F AS patients carry at least one of low expressed allele.

These findings support the hypothesis that KIR3DL1 is not simply a passive counterpart of the segregating KIR3DS1 allele product but play a protective role, although the presence of activating KIR3DS1 allele is more important for AS susceptibility. However individuals carrying two high level expressed inhibitory KIR3DL1*F alleles can be affected by AS.

P09.137

Predicting development of canine leishmaniasis using genomic selection analysis from genome-wide SNP data

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Leishmaniasis affecting humans and dogs constitute a public health and veterinary problem. Leishmaniasis development upon infection from *Leishmania* depends partly on host genetic factors. Genetic-based diagnosis would be a useful tool and therefore we sought to predict affection status in dogs using genomic selection from genome-wide SNP data.

Affection status was defined as healthy infected (>4 years and prior infection, *n*=115) or affected (diagnosed before 4 years, *n*=104). Samples were from Boxer dogs, a breed with higher susceptibility to leishmaniasis. Samples were genotyped with the Illumina's CanineHD and 99,997 SNPs were left for analysis after data cleaning. A modified BayesB method was used under three different models varying on whether covariates were fitted. This produced estimates of markers and covariates effects which were used to calculate predictions for the affection status.

First, the correlation (*r*) between predictions and actual phenotypes was used as measure of predictive power. It was higher in the unpermuted

dataset than in any of 10 permuted sets regardless of the model. When predictions from five cross-validation sets were combined, the highest *r* was obtained for Model-3 and it was greater than for Models 1-2. Second, the area under the ROC curve (AUC) was used to compare sensitivity and specificity, with Model-3 performing better than Models 1-2. Finally, a threshold of 1.5 would produce the highest fraction of correctly diagnosed patients.

	Model-1	**Model-2**	**Model-3**
Covariates	-	Genetic stratification	Genetic stratification Lifestyle
Unpermuted			
r	0.98	0.98	0.98
CI	(0.98, 0.99)	(0.98, 0.99)	(0.97, 0.98)
P-value	$2 \cdot 10^{-16}$	$2 \cdot 10^{-16}$	$2 \cdot 10^{-16}$
Permuted (10 sets)			
r_{avg}	0.92	0.82	0.77
sd	0.02	0.06	0.07
P-value	$2 \cdot 10^{-16}$	$2 \cdot 10^{-16}$	$2 \cdot 10^{-16}$
Cross-validation (5 sets)			
r	0.18	0.20	0.51
CI	(0.05, 0.30)	(0.07, 0.32)	(0.41, 0.61)
P-value	< 0.01	< 0.01	$< 10^{-15}$
AUC	0.62	0.63	0.80
$g_{1.5}$	0.62	0.60	0.72

P09.138
Association of multiple variants along 6p21.3 region with leprosy susceptibility in two unrelated population groups of India

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Leprosy is a chronic infectious disease caused by Mycobacterium leprae which affects mainly skin and nerve of the host and results in characteristic deformity and disability. Host genetic factors strongly determine susceptibility to leprosy and its subtypes. We studied the selected chromosomal region 6p21.3, which comprises of functionally important genes involved in immune response against M. leprae. Status of 237 SNPs (111 SNPs with MassArray, 120 SNPs with Allele-specific PCR Kit and 6 with Sequencing) within HLA class I, II and III region in 2592 individuals, representing two unrelated population groups, was assessed. The disease associated SNPs were filtered in multiple steps of analysis, either on the whole sample size or a representative sample set, sufficient for statistical and LD analysis. Eight SNPs within BAT1 promoter (rs2523504, p=2.5E-06); exon 3 of NFKBIL1 (rs2230365, p=1.9E-08); LTA 13kb upstream (rs13192469, p=7.2E-07); LTA promoter (rs36221459, p=1.7E-05); TNF intron1 (rs1800610, p=2.8E-4); TNF-LTB downstream (rs769178, p=5.8E-05) and BTNL2-DRA interval (rs3135365, p=4.6E-24 and rs7773756, p=3.3E-18) were significantly associated even after adjustment with sex and the boneferroni correction for multiple testing. In addition to find the role of previously unidentified genes, BAT1 and BTNL2, we also established the functional status of the SNPs in in-vitro reporter assays. Further, an assessment of an interaction of multiple genes in unison within the studied region provided a graded risk to leprosy depending on the combination of genotypes for the significantly associated functional SNPs. These observations dissect the role and help in better understanding of HLA loci involved in disease pathogenesis.

P09.139
High-throughput omics approach provides clues for novel pathways behind low HDL-cholesterol

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Low HDL-cholesterol is a major risk factor for cardiovascular disease. To elucidate potential novel pathways behind low HDL-C, we have employed 3 different high-throughput omics: genomics, transcriptomics, and lipidomics.
We have performed GWAS on 450 Finnish individuals with low or high HDL-C (10th and 90th population percentiles). Out of subset of these individuals (n=54), we obtained subcutaneous fat biopsies for genome-wide expression analysis, and isolated plasma HDL particles for lipidomics analysis with MS technology.
We first performed gene network analysis for genetic loci associated with low HDL-C observing that SNPs within four inflammatory pathways (e.g. antigen-presentation) were enriched among the low HDL-cholesterol associated genes (p=10^-5). Also, these inflammatory pathways were over-expressed in the adipose tissue of low-HDL-subjects. We then calculated genetic risk scores based on low HDL-cholesterol associating SNPs from these pathways, observing that high risk score resulted in not only decreased HDL-cholesterol-levels but also increased expression of these inflammatory pathways. Moreover, individual genes of these pathways (e.g. HLA-DRB1) exhibited allele specific expression, inversely correlating with HDL-C-levels.
Consistent with enrichment of inflammatory processes in association and expression analyses, the inflammatory nature of the HDL particle itself was evident in the lipidomics analysis. For instance, ceramides with well-established pro-inflammatory function were elevated in HDL-particles from subjects with low HDL-C, and anti-oxidative ether-bond containing plasmalogens down-regulated.
Our findings imply that genetic variation influences the molecular alterations associated with low HDL-cholesterol on both expression and metabolite levels, suggesting a more inflammatory and less vasoprotective role for HDL particles in subjects with low HDL-C.

P09.140
LRRK2 Mutations in sporadic and familial Parkinson disease

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Parkinson disease (PD) is a chronic and progressive neurodegenerative disorder characterized by 4 cardinal signs: resting tremor, bradykinesia, rigidity and postural instability. Monogenic forms of PD account for only a small percentage of the affected population (<10%). The majority of PD cases are considered to be caused by the interaction of genetic and environmental factors, and susceptibility variants of genes involved in monogenic forms of PD have been identified in sporadic PD in several populations. Recently, mutations in the LRRK2 gene have been implicated in familial dominant and sporadic cases of PD. The G2019S mutation in the LRRK2 gene has been identified in approximatelly 5-6% of familial forms of PD and about 1-2.5% of sporadic cases, and constitutes the most frequent genetic cause of PD known to date. In this study, 6 mutations in the LRRK2 gene were determined in 326 patients with PD and 135 control subjects. The mutations analyzed were G2019S, R1441C/G/H, I2020T and Y1699C. Eleven of 326 patients with PD carrying the G2019S mutation (3.4%). None of the other LRRK2 mutations were found in the remaining patients. The phenotype of the 11 G2019S mutation carriers were similar to those of typical PD. The age of onset was variable, ranged from 37 to 80 years. In conclusion, the G2019S mutation frequency in PD patients was similar to that reported in other European countries, and this mutation was not identified in controls. The R1441C/G/H, I2020T and Y1699C mutations were not found in any of the PD patients or controls.

P09.141
Can mutations in LTBP2 cause Pseudoexfoliation?

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Pseudoexfoliation (PEX) syndrome is a common age-related elastic microfibrillopathy, characterized by deposition of fibrillar extracellular material (PEX material) in the eye, particularly in the anterior segment. PEX is sometimes accompanied by glaucoma, and is considered

the most common cause of secondary glaucoma. Pseudoexfoliation glaucoma (PEXG) may be caused by hindrance of drainage of aqueous humor caused by PEX material. PEX deposits consist of complex cross linked fibrils including components of elastic microfibrils such as fibrillin1 and Latent TGFβ Binding Protein 2 (LTBP2). The genetic basis of PEX remains largely unknown. An association between PEX and a few nucleotide variations in the LOXL1 gene coding lysyl oxidase-like 1 have been reported in some populations. However, the data on LOXL1 from different populations are not all consistent and the role of the gene with respect to PEX is unclear.

LTBP2 was recently identified as a primary congenital glaucoma causing gene. The protein product of the gene may have structural and non-structural functions. As glaucoma often occurs along with PEX, and as LTBP2 is a component of PEX deposits, we queried the possibility that mutations in LTBP2 may cause or predispose individuals to PEX.

Exonic regions of LTBP2 were sequenced in 47 PEX affected individuals. In addition to several previously reported sequence variations, five novel coding variations that cause amino acid changes were observed in the DNA of the patients. At least one of the variations that affects a proline residue is quite likely to be associated with the PEXG phenotype.

P09.142
Copy number variation in 16p11.2 is associated with major depressive disorder

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Major depressive disorder (MDD) ranks among the top ten causes of global burden of disease. To our knowledge, only one systematic CNV screening of patients with MDD has been carried out. We performed a genome-wide analysis of CNVs in 575 German patients with a DSM-IV diagnosis of MDD and 1,618 controls. We genotyped all individuals using either Illumina's HumanHap550, Human610 or Human660W arrays. To identify potential CNVs, we analyzed each individual's SNP-chip information with QuantiSNP and PennCNV. To minimize the number of false-positive CNV calls, we developed a stringent quality protocol, requiring CNVs to have a minimum of 30 consecutive SNPs and a confidence value of at least 30. The two datasets generated by QuantiSNP and PennCNV were analyzed separately. Only those regions for which both programs generated nominal significant P-values were included in further analyses.

We discovered a significant overrepresentation of CNVs in four different chromosomal regions (7p21, 15q26, 16p11.2, 18p11) in patients compared to controls. To our knowledge, CNVs in 7p21, 15q26, and 18p11 have not been described in MDD or other psychiatric disorders. The finding on chromosome 16p11.2 is of particular interest as CNVs in this region are associated with autism and schizophrenia. In our sample we observed three rare CNVs in 16p11.2 (one microdeletion, two microduplications) in patients and none in controls (P = 0.0178).

Our study provides evidence for an involvement of CNVs in the development of MDD and indicates an extension of the phenotype associated with CNVs in 16p11.2.

P09.143
Relationship between IKBKE gene and depression and panic disorder

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Major depressive disorder (MDD) and panic disorder (PD) belong to the most prevalent mental diseases, affecting 10% and 3% of general population, respectively. Alterations in immune system have been implicated in the onset and development of MDD and PD. We studied the relationship between single-nucleotide polymorphisms (SNPs) of IKBKE (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon) gene from chromosomal region 1q32 and MDD and PD. The association study design was used: 17 SNPs covering the IKBKE gene were analyzed in 581 unrelated patients and in 389 healthy control subjects. All subjects were individuals of Caucasian origin living in Estonia. Patients were divided into two groups according to diagnosis: comparison of allelic and haplotypic frequencies was performed between control group and MDD patients (n=391), PD patients (n=190), and the whole patient group (n=581). Both MDD and PD groups included 'pure' phenotypes as well as phenotypes comorbid to other mood and anxiety disorders. SNPlex Genotyping System was applied for genotyping, following association and haplotype analyses with Haploview program. Association analysis revealed the most prominent relationship between MDD patient group and SNPs rs1930437 and rs2274902 (allelic p values 0.0007, 0.0013, respectively). Statistically significant differences occurred in the panic and whole group as well. Haplotype analysis revealed five haplotype blocks in all tested groups of patients compared to healthy controls. Significant haplotypic associations confirmed allelic associations in MDD group. These results suggest that IKBKE gene from 1q32 chromosomal region may possibly be related to mood and anxiety disorders.

P09.144
Variants associated with mammographic density are also associated with breast cancer

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Introduction: Mammographic density (MD), is one of the strongest breast cancer risk-factors. However, the mechanism by which this risk is mediated is unclear. Recent genome-wide association studies (GWAS) have demonstrated that a variant in ZNF365 is associated with both breast cancer risk and MD[1;2]. We investigated if other SNPs showing evidence for association with MD in the meta-GWAS were associated, in aggregate, with breast cancer risk.

Method: The SNPs associated with MD were identified through meta-analysis of 5 GWAS(n=4877). For each SNP, Z-statistic for combined effect over all studies was computed and converted into per-allele effect size estimate (β_k). Effect of each SNP on breast cancer risk was then assessed using data from UK2-GWAS study (3628/5190). The combined effect of multiple SNPs was evaluated by multiplying signed MD beta value(β_k) for each of *N* SNPs over given significance threshold in MD meta-GWAS by their number of effect alleles at that SNP, G_{ik}(0, 1 or 2), and summing over all *N* SNPs to create the individual's score($\mathbf{Z}_i$).

$$\mathbf{Z}_i = \sum_{k=1}^{N} \beta_k \, G_{ik}$$

Logistic regression was then used to examine if the individual's score was predictive of breast case-control status.

Results: The top 5% of density SNPs significantly predicted breast cancer case-control status in the UK2-GWAS(P=0.005 & P=0.009 genotyped & imputed SNPs respectively). This predictive effect was not seen when only top 1% of density SNPs were used(P=0.3 & P=0.5 using genotyped & imputed SNPs respectively).

Conclusions: These results suggest that many SNPs associated with MD are also associated with breast cancer.

P09.145
Association between the SNAP25 gene and the extremes in intellectual performance.

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Introduction: Intelligence in the normal and high range is shown to be highly heritable. Low intelligence, mental retardation, is thought to have a heritable cause in about 50%. Association studies have identified some loci and genomic variants associated with variation in intelligence in the normal range. High IQ is thought to be caused by the additive effect of the same genomic variants, but experimental evidence for this hypothesis is lacking. For mental retardation however, known genetic causes are rare and monogenetic.

Aim: We set out to test whether the SNAP25-gene that was previously linked to intelligence in the normal range, is also linked to variation in intelligence at both the lower (mental retardation) and the upper range (high IQ).

Methods: We genotyped two SNP's in SNAP 25 (rs363039 and rs363050), previously linked to variation in normal intelligence (Gosso, et.al. 2006), in a cohort of 644 children with mental retardation and a cohort of 360 participants of the Amsterdam growth and health study, which all were following the highest level of education at the time of recruitment.

Results: We found a significant (p>0.05) lower allele frequency of the allele associated with an higher IQ (gosso et. al.) for both SNP's (rs363039 and rs363050) in SNAP25 in the MR cohort compared to the high IQ cohort. The minor alleles of both SNP's are associated with mental retardation.

Conclusion: Our results suggest that, at least for SNAP-25, the same genomic variants affect both variation in normal or high intelligence and mental retardation.

P09.146
COL1A1 association and otosclerosis: a meta-analysis

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Otosclerosis is a disease of abnormal bone remodeling in the human otic capsule that can lead to progressive hearing loss. Little of the underlying disease etiology has been elucidated thus far, although several studies have suggested that COL1A1 may play a role based on its importance in bone metabolism and other diseases like osteoporosis and osteogenesis imperfecta. Genetic association studies between COL1A1 and otosclerosis, however, have been contradictory. To resolve this issue, we studied a large Belgian-Dutch and a Swiss population for a genetic association between COL1A1 and otosclerosis and additionally performed a meta-analysis to investigate the overall genetic effect of COL1A1 on all otosclerosis populations studied to date. We found a significant association both in the Belgian-Dutch population and in the meta-analysis. In aggregate, our analysis supports evidence for an association between COL1A1 and otosclerosis, although effect sizes of the variants reported in the initial studies are likely to be an overestimate of true effect sizes.

P09.147
A meta-analysis of thyroid-related traits reveals novel loci in the regulation of circulating thyroid stimulating hormone and thyroxine circulating levels

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Thyroid stimulating hormone (TSH) and thyroxine (T4) are key regulators of thyroid function, growth and metabolism. Their dysregulation leads to common endocrine disorders. Despite being relatively highly heritable (65% and 40% respectively), genetic loci so far identified only account for a small proportion of the estimated genetic variation. To identify additional associated regions, we carried out a meta-analysis of up to 12 GWAS using ~2.5 million directly genotyped or imputed autosomal SNPs, corresponding to 18,538 and 13,590 euthyroid subjects of European ancestry for TSH and FT4, respectively. Standard quality filters were applied to each study, and single marker association estimates were combined using inverse-variance weights. For TSH, we confirmed association at PDE8B (rs1382879 p=1.28x10-32) and CAPZB (rs10799824, p=1.0x10-16), and observed 7 novel loci at chr6q27, chr2q35, chr4q31.23, chr9q34.2, chr16q23.2, chr6p21.1 and chr15q26.1 (p<5x10-8). For FT4 we confirmed association at DIO1, for which there was suggestive evidence from candidate gene studies, and observed 2 additional signals at 9q34.3 and 4q33. At 6 genes, including PDE8B, evidence for heterogeneity (p<0.05) was observed, and at 4 of which could be explained by different effects between males and females (p<10-4). We also carried out gender-specific association analyses, but no additional genes were identified. Preliminary analyses on individuals with extreme phenotypic values suggested that TSH associated variants may contribute to thyroid pathologies. Overall, 10 and 3 independent SNPs for TSH and FT4 respectively, explain 3.9% and 1.3% of the heritability, suggesting that several additional loci, remain to be discovered.

P09.148
Genome-wide screen for metabolic syndrome loci

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Dyslipidemia, hypertension, insulin resistance, and measurements of obesity are key components in definition of the metabolic syndrome (MetS). Genome-wide association studies (GWASs) have identified several susceptibility loci for these MetS component traits, but there is little evidence for genes contributing to the syndrome as an entity rather than to individual component traits.

We conducted a GWAS on MetS and its component traits in four Finnish cohorts (2,637 MetS cases and 7,927controls), and followed the top loci in an independent sample with transcriptome and NMR-based metabolome data. Furthermore, we tested for loci associated with multiple MetS component traits using factor analysis and built a genetic risk score for MetS risk.

A previously known lipid locus, *APOA1/C3/A4/A5* gene cluster region, was associated with MetS in all four study samples (meta-analysis P=7.23x10^{-9}). Primary source of the association was the SNPs effect on triglyceride (TG) (P=2.59x10^{-31}) and high-density lipoprotein (HDL) levels (P=5.83x10^{-8}), which was further supported by serum metabolite analysis, where *rs964184* associated with various VLDL, TG, and HDL metabolites (P=0.024-1.88x10^{-5}). 22 previously identified loci for individual MetS component traits were replicated in our GWAS but none of these loci significantly associated with two or more uncorrelated traits. A genetic risk score (GRS), calculating the number of alleles in loci associated with individual MetS traits, was strongly associated with MetS status.

Our findings suggest that MetS does not have a common genetic background but is likely to result from complex interactions between lifestyle and multiple genetic variants which in combination may predispose to MetS.

P09.149

Metabolic and genetic cardiovascular risk factors in manifestation of metabolic syndrome in males.

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Metabolic syndrome (MS) is a cluster of metabolic and genetic entities closely associated with the development of cardiovascular disease (CVD) and diabetes mellitus type II (DM2). Male sex is an important factor in the pathogenesis, risk, and prognosis of many known diseases. Therefore, the aim of this study was to investigate the most significant cluster of cardiovascular risk factors specific to males. Patients and methods. The MS patient group consisted of 176 males with abdominal obesity, having at least two of the CVD risk factors (average age 61.7Â±1.0). Among them, 89 patients had DM2, 20 patients had DM2 and survived after myocardial infarction, and 67 patients survived after myocardial infarction but did not have DM2. The control group consisted of 115 males without CVD and DM2 (average age 40.0Â±0.5). Polymorphisms of genes APOA1 G-75A and C+83T, APOC3 Sst1, APOE, APOA5 T-1131C and S19W, ADRB3 W64R, and ACE I/D were studied. Statistical analysis included logistic regression method. Results. We have found cardiovascular risk factors associated with manifestation of MS in males. Factors that increased the probability of MS in males were moderate and severe obesity, and the 19W-allele of the gene APOA5. The normal level of triglycerides appeared to have protective effect. Conclusion. It is evident from the study that the normal triglyceride levels are protective against MS and CVD in males. And the 19W-allele APOA5 that was previously shown to associate with elevated triglyceride levels is the MS risk factor.

P09.150

Genetic variations in tumor necrosis factor alpha, interleukin 10 genes and migraine susceptibility

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Migraine is a very common headache disorder and pathogenesis of the disease is still unknown. Cytokine genes have been implicated in migraine susceptibility. The present study was designed to find out whether polymorphisms in the tumor necrosis factor-α (TNF-*α*), interleukin-10 (IL-10) gene and interleukin-10 haplotypes are associated with the risk of migraine.

We analyzed TNF-*α* -308G/A, IL10 -1082G/A, -819C/T, and -592C/A polymorphisms in 203 migraine patients and 202 healthy subjects by using amplification refractory mutation system-polymerase chain reaction.

The -308G/A genotypic and -308A allelic frequency of TNF-α polymorphism was higher in migraine patients than healthy controls and significant association was found between migraine and TNF-α -308G/A polymorphism (Pc: <0.000, OR: 2.16, 95% CI: 1.44-3.28). No statistically significant association was found between IL10 -1082G/A, -819C/T, and -592C/A polymorphisms and haplotypes containing these alleles and migraine.

Our results reflect that TNF-α -308G/A polymorphism may be one of the many genetic factors for migraine susceptibility in Turkish population.

P09.151

Mitochondrial DNA variants (7086 C/haplogroup H/D310 >7C) increased the risk for new-onset diabetes after transplantation

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Background: New-onset type 2 diabetes (DM2) is a frequent complitation among transplanted patients treated with the immunosuppressor tacrolimus (Tac). Several gene variants associated with the risk DM2 in the general population would also increase the risk of posttransplant DM2 (NODAT). Common mitochondrial DNA variants (haplogroups) were also associated with DM2. We investigated the effect of nine mtDNA polymorphisms on NODAT among heart or kidney transplanted patients treated with Tac.

Methods: The nine mtDNA SNPs that define the common European haplogroups, and the 310 polyC and T16189C SNPs were determined in 120 NODAT and 200 patients who remained non diabetics (no-NODAT) from the same region (Asturias, Northern Spain).

Results: Mitochondrial haplogroup H (7028C) was significantly more frequent in the NODAT group (56% vs. 35%; p=0.001; OR=2.38, 95%CI=1.48-3.72). The mtDNA 16189 SNP was not associated with NODAT-risk. The NODAT group had a higher frequency of D310 >7 C repeats (70% vs. 60%; p=0.012). Haplotype 7028-C/D310- 7C would have a protective effect against the development of NODAT. Sequencing of the mt HVI region from blood leukocytes showed that this haplotype was linked to a lower D310 polyC heteroplasmy compared to the other haplotypes.

Conclusion: Common mtDNA variants were associated with the risk of NODAT. This effect could be due to the higher instability of mtDNA replication among individuals with at risk haplotypes, and this could affect mitochondrial function in beta-pancreatic cells in response to Tac increasing the risk for DM2.

P09.152

A novel m.3395A > G missense mutation in the mitochondrial ND1 gene associated with the new tRNAIle m.4316A > G mutation in a patient with hypertrophic cardiomyopathy and profound hearing loss

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Mitochondrial diseases are a clinically heterogeneous group of disorders that arise in young and adult patients at any age, as a result of dysfunction of the mitochondrial respiratory chain. Mitochondrial dysfunction frequently affects the heart and may cause both hypertrophic and dilated cardiomyopathy. The cardiomyopathy is usually a part of a multisystem involvement and may rarely be isolated. In fact, it can be associated with others disorders, such as hearing loss. The state may be stable for many years, but rapid deterioration may occur and often lead to congestive heart failure.

We described a newborn girl with hypertrophic cardiomyopathy and profound hearing loss. The mtDNA mutational analysis revealed the presence of known polymorphisms associated to cardiomyopathy and/or hearing loss, and 2 novel heteroplasmic mutations: m.3395A > G (Y30C) occurring in a highly conserved aminoacid of the ND1 gene and the m.4316A > G located in the residue A54 of the tRNAIle gene. These 2 novel variations were absent in 150 controls. All these variants may act synergistically and exert a cumulative negative effect on heart function to generate the cardiomyopathy.

P09.153

Genome-wide association study of myxomatous mitral valve disease in Cavalier King Charles Spaniels identifies two candidate loci

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Myxomatous mitral valve disease (MMVD) is a degenerative heart disease with a reported incidence in humans of 1-5%. The disease also has a high prevalence in dogs, especially in Cavalier King Charles Spaniels (CKCS), of which, nearly all are affected at the age of 10 years. In affected individuals the mitral valves become enlarged, lose flexibility and eventually protrude into the left atrium, resulting in mitral regurgitation (MR). Since dog breeds are much more homogeneous than the human population it is more feasible to indentify genetic components underlying complex diseases in dogs. Thus, dogs provide good models for human diseases. We performed a genome-wide association study (GWAS) in CKCS to identify the genes underlying MMVD. Cases and controls were selected using stringent criteria: cases were dogs with early onset of MMVD i.e. murmur ≥1 and MR jet >20% at <4.5 years, or with clinical signs of heart failure; controls were dogs with late onset i.e. murmur ≤2 and MR jet <50% at >8 years. A total of 139 cases and 102 controls were genotyped with the 170k CanineHD BeadChip (Illumina). Genome-wide association analysis using PLINK was performed, and the resulting *p*-values were adjusted for multiple testing. The GWAS revealed two statistically significant regions on canine chromosomes 13 and 14 (*p*-values: $4.0 \cdot 10^{-5}$ - $4.7 \cdot 10^{-2}$ and $7.9 \cdot 10^{-4}$ - $1.7 \cdot 10^{-2}$, respectively). Thus, we have identified two novel candidate regions, in which genetic components of importance for MMVD are located. Studies are currently underway to identify the causative mutations within the two regions.

P09.154

Somatic Copy Number detection in MZ twins discordant for Congenital Diaphragmatic Hernia (CDH) or Esophageal Atresia (EA) and in affected diaphragm or proximal pouch tissue

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The occurrence of phenotypic differences between monozygotic (MZ) twins is commonly attributed to environmental factors, assuming that MZ-twins have a 100% identical genetic make-up. Yet, recently several lines of evidence showed that both genetic and epigenetic factors could play a role in phenotypic discordance after all. One report suggested a high occurrence of low-mosaic CNVs within MZ-twin pairs discordant for Parkinson disease, thereby stressing on the importance of post-zygotic mutations as disease-predisposing events.

In this study, we analyzed the prevalence of discrepant somatic CNVs in discordant MZ-twins of the Esophageal Atresia and Congenital Diaphragmatic Hernia cohort in Rotterdam. We used high-resolution SNP-arrays on blood- derived DNA from 11 (4 CDH and 7 EA) pairs of MZ-twins. Paired analysis was executed with specific attention to isolated allelic imbalances as a marker for low-mosaic CNVs. In this way, we showed that somatic CNVs are not the cause of phenotypic discordance in this cohort of MZ-twins. In addition, bead chip genotyping showed no evidence for pathologic SNP differences within each MZ-pair either. To exclude the possibility of somatic mutations restricted to the affected tissues, we screened for CNVs in proximal pouch- and diaphragm tissue of a separate group of patients as well. In line with the twin data, no target-tissue specific CNVs were revealed in DNA from 12 pouch- and 13 diaphragm biopsies. Yet, recurrent, germ-line chromosome 22q11-13 were discovered. In conclusion, post-zygotic structural mutations are not a common cause of OA and CDH.

P09.155

The investigation of TNF alpha 308G/A polymorphism in mood diseases

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The pathogenesis of mood diseases is still unclear; However an increasing amount of evidence suggests that an imbalance of inflammatory cytokine activity may be a contributing factor in etiology and pathophysiology of mood disorders. Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine with functions in nerve cell growth, differentiation, synaptic scaling, and apoptosis in the central nervous system. Thus TNF-α is considered a plausible candidate gene for mood diseases. The level of TNF-α production in under genetic control and is thought to be influenced by a -308G/A promoter polymorphism. In this study we aimed to investigate the plausible association between TNF-308G/A polymorphism and major depression (MD) and bipolar disorder (BD). We analyzed TNF-308G/A polymorphism in 72 MD, 76 BD patients and 299 healthy subjects by using amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). A significant association was found between TNF-308G/A polymorphism and BD but there are no correlation between MD and this polymorphism (Table 1). Our findings suggest that TNF-308G/A polymorphism may play an important role in susceptibility to BD in Turkish patients.

Table 1. Distribution of TNF-308G/A polymorphism in mood diseases and control

TNF locus n= (%)	**BD** 76	**MD** 72	**Control** 299	P OR: (95%CI)
308G/A				[a] 0.000 3.65 (2.15-6.18)
A/A	0	0	0	[b] 0.117
G/A	39 (51%)	21 (29%)	67 (22%)	
G/G	37 (49%)	51 (71%)	232 (88%)	
Allelic frequency	39 (26%)	21 (15%)	67 (11%)	
A	113 (74%)	123 (85%)	531 (89%)	[a] 0.000 2.74 (1.70-4.35)
G				[b] 0.133

[a]: p value (BD vs controls), [b]: p value (MD vs controls)

P09.156
Somatic mosaicism for FII G20210A and novel FII T20061C polymorphisms in patient with reccurent pulmonary thromboembolism

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Mosaicism implies the presence of more than one genetically distinct cell line in a single organism. Although it is frequently masked, somatic mosaicism has been implicated in more than 30 monogenetic disorders. Possible role of mosacism in multifactorial diseases is still unknown. Thrombophilia is a multifactorial disorder, involving both genetic and acquired risk factors that lead to increased tendency to thrombosis. The most frequent thrombophilic genetic risk factors are FV Leiden and FII G20201A gene variants.

Here we describe a female patient (48 years) presented with recurrent pulmonary thromboembolism and pulmonary artery hypertension. Routine thrombophilia screening was performed six months after thromboembolic episode. Obtained results were within normal range with exception of conflict results for FII G20210A gene variant. In order to elucidate these results we performed sequencing of 715bp segment of 3`end of prothrombin gene using Applied Biosystems 3130xL Genetic Analyzer. Sequence analysis of DNA from buccal swab cells confirmed presence of heterozygous G20210A gene variant, as well as new C to T transition on 20061 nucleotide. No sequence abnormalities in this region of prothrombin gene were detected in blood and hair bulb cells DNA.

In conclusion, this is the first report of mosaicism for FII G20210A polymorphism and its' possible association with thrombophilia. Current knowledge of potential role of mosaicism in pathogenesis of multifactorial diseases other than cancer is very limited and this area requires further studies.

P09.157
MSX1 gene, as a candidate gene, is not a rik factor for non-syndromic cleft lip and palate formation in Turkish population

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Introduction: Nonsyndromic cleft lip with/without palate (NS-CL/P) affects about 1/1000 livebirths with wide variability concerning geographic distribution, ethnic background and socioeconomic status. Gene-gene and gene-environmental interactions have been implicated in NS-CL/P. Studies in mice and humans supported the MSX1 as a strong candidate gene in recent years. But most of the results are contradictory including both case- control and parametric linkage studies. In this study, we aimed to analyze the sequence variations of MSX1 to determine MSX1 mutations that might be etiological to patients with NSCL/P in Turkish population.

Material and Methods: 100 controls and 100 patients without any family history of NS-CL/P were recruited to the study. Mutation analyses of the MSX1 gene was carried out by PCR- SSCP- Sequencing methodology.

Results: According to the SSCP and sequencing analyses, we couldn't detect any sequence variations in patients and control groups.

Conclusion: Our study was based on population- based and case-control design, not case- parent design because of having no information about MSX1 gene alterations in Turkish population. MSX1 gene variants were linked to the NS-CL/P formation in some populations but the same results couldn't be obtained for other populations. In order to clarify the role of MSX1 gene, different population- based studies are needed. Due to our results, MSX1 gene seems not to have target role in NS-CL/P formation, at least alone. More studies including the same and the other gene variation analyses should be carried out inorder to determine the roles of related genes in Turkish population.

P09.158
Study of association of common haplogroup-defining mtDNA polymorphisms with different multifactorial diseases in Russian population

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Mitochondrial genes encode subunits of respiratory chain complexes which are involved in fundamental cell function. Thus, mtDNA polymorphisms may contribute to susceptibility to common diseases, and there is some evidence for this, mostly for neurodegenerative conditions. It should be noted that genome-wide association studies omit mtDNA because of non-Mendelian inheritance. We have studied patients with multifactorial diseases of different etiology: cardiovascular (coronary heart disease, N=175), allergic (asthma, N=150), and infectious (hepatitis C, N=182; tuberculosis, N=214). Population sample (N=424) has been studied for comparison. All samples were collected in Tomsk (West Siberia) from individuals of Russian origin. Common haplogroup-defining SNPs in mtDNA were genotyped (C7028T for haplogroup H, A12308G for U, A15607G for T, and G13708A for J). In the population, the SNPs frequencies corresponded to those known for most European populations, i.e. H: 38.68%, U: 24.76%, T: 9.91%, J: 7.31% . We have found higher frequency of haplogroup J in asthma (15.33% in the patients; χ^2=7.45; P=0.0063; OR=2.3 with CI 1.24-4.23). The JT cluster also has shown association with asthma (26.67% vs 17.22% in the population; χ^2=5.8; P=0.0160; OR=1.75 with CI 1.1-2.78). In addition, haplogroup T frequency was significantly lower in the hepatitis C group (3.43%; χ^2=6.2; P=0.0128; OR=0.35 with CI 0.15-080). In the samples with coronary heart disease and tuberculosis, no associations have been revealed. Our results suggest that mtDNA polymorphisms may play some role in susceptibility to allergic and infectious diseases, while connections between their pathogenesis and mitochondrial functions are not obvious.

P09.159
C677T polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene, systolic blood pressure and echocardiographic left ventricular parameters in children with systolic arterial hypertension (AH)

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Background. Hyperhomocysteinemia is a risk factor for cardiovascular disease. C677T polymorphism of the MTHFR gene may account for increased plasma levels of homocysteine.

Objectives: To investigate distribution of MTHFR genotypes frequency of C677T polymorphism in children with systolic AH. To compare clinical systolic BP levels and values of left ventricular parameters between carriers of various genotypes. **Patients:** 85 children aged 5-17 with systolic AH were studied.

Methods. C677T polymorphism of MTHFR gene was detected with PCR-RFLP. LV mass (LVM), LV mass index (LVMI), interventricular septum thickness in diastole (IVSd), LV posterior wall in diastole (LVPWd), LV end diastolic diameter (LVEDD), and relative wall thickness (RWT) were assessed by echocardiography.

Results. There were identified 21(25%) C/C homozygotes, 55(66%) C/T heterozygotes and 7(8%) TT homozygotes. We have found a significant differences in systolic BP levels and RWT values between carriers of different genotypes. Clinical systolic BP in patients with CC genotype was 135,24±8,7 mm Hg, CT genotype was 131,95 ±10,4 mm Hg, and TT genotype was 131,85±3,3 mm Hg (p<0,05). RWT in patients with CC genotype was 0,36±0,06, CT genotype was 0,34±0,05, and TT genotype was 0,32±0,04 (p<0,05). No significant difference was observed in other echocardiographic LV parameters

among subjects carrying the C/C, C/T, T/T genotypes.
Conclusion. We suggested that the C677T polymorphism of MTHFR gene may be involved in AH and the LV remodeling process.

P09.160
MTHFR C677T and A1298C polymorphism in Iranian patients with migraine

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Introduction: Migraine is a common neurologic disorder and familial clustering raises the probability that genetic factor might play a role in migraine susceptibility. Methylene tetra hydrofolate reductase (MTHFR) is a key enzyme in folate metabolic pathway and is suggested to be related to migraine susceptibility. However there are conflicting data on the association between the MTHFR polymorphisms and disease. This study performed to find the association between the MTHFR 677C>T and A1298C polymorphism and migraine.
Material and Methods: Migrainous patient according to the international headache society criteria included in the study. Healthy people without any positive history of periodic headache and no family history of migrainous headache in the family recruited as control group. MTHFR C677T and A1298C polymorphisms were investigated in cases and controls using PCR-RFLP method.
Results: Seventy-five migrainous patient (18 males and 57 females) and 128 healthy controls (43 males and 85 females) were recruited in the study. MTHFR 677TT was more frequent in migrainous patients (17.1% vs, 3.1%, P<0.05) and were associated with higher risk of migraine (OR= 6.5, CI 95%; 2.03-20.76).
Conclusion: It seems MTHFR polymorphism may predict the susceptibility to migraine attach. More studies with more sample is needed in Iranian population.

P09.161
Cytokine genes polymorphism and susceptibility to multiple sclerosis in the republic of Bashkortostan

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Multiple sclerosis (MS; MIM 126200) is a demyelinating autoimmune disease of the central nervous system caused by interplay of environmental and genetic factors. Cytokines play an important role in MS pathophysiology, and genes encoding various cytokines are logical candidates to assess possible associations with MS susceptibility and disease course. The study was aimed to evaluate the associations of MS with polymorphisms of tumor necrosis factor alpha gene (*TNFA*, -308 G>A), lymphotoxin alpha gene (*LTA*, 252A>*G),* interleukin 6 gene (*IL6*, -572G>C*)*, interleukin 10 gene (*IL10*, -627C>A, interleukin 12 gene *(IL12B*, -1159A>C) in Russians and Tatars by ethnic origin living in Ufa of the Republic of Bashkortostan, Russia.
DNA was amplified in polymerase chain reaction in 265 patients with MS and 633 controls. It has been revealed that genotype *TNFA*(-308)*G/*G (P=0.045, OR=0.047, CI 0.23-0.97), genotype *LTA*(252)*A/*A (P<0.001, OR=0.31, CI 0.16-0.59) are associated with a decreased risk of the disease development in female from ethnic Tatar, but genotype *LTA*(252)*A/*G is associated with an increased risk of MS (P=0.006, OR=2.62, CI 1.35-5.08). For Russian male genotype *IL6*(-572)*G/*G (P=0.001, OR=12.55, CI 1.68-93.7) is associated with an increased risk of MS, but genotype *IL6*(-572)*G/*C (P=0.003, OR=0.09, CI 0.01-0.67), genotype *IL12B*(-1159)*C/*C (P=0.048, OR=0.12, CI 0.02-0.89) can be considered as a marker of decreased risk for MS. The association of *-627C/A* polymorphism of *IL10* gene with MS has not been detected in both ethnic groups.
Our results suggest contribution of investigated cytokine genes polymorphism in susceptibility to MS in Russians and Tatars.

P09.162**
Do ST-segment elevation and non-ST-segment elevation myocardial infarction have different genetic backgrounds?

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The most robustly replicated common risk allele for myocardial infarction (MI) at 9p21.3 affects gene expression rather than the structure of a protein. This is consistent with the idea that differences in gene expression might explain a significant part of the genetic risk for common diseases. It is also believed that the genetic background differs for different subcategories of complex phenotypes. Taken together, this led us to test two hypotheses: First, many common expression quantitative trait loci (eQTL) are collectively associated with MI. Second, the effects of these loci are not equal for two subtypes of MI, namely ST-segment elevation MI (STEMI) and non-ST-segment elevation MI (NSTEMI).
We performed a GWAS in a sample consisting of acute coronary syndrome patients and healthy controls (n=3373). We selected 20,092 SNP's annotated as eQTL's in a public database and compared them with SNP's randomly selected from the dataset by permutation.
The set of eQTL SNP's is associated with MI (p<0.001). This effect is significantly stronger for NSTEMI (p<0.001) as it is not seen in STEMI (p=0.616). The difference is driven by eQTL SNP's at the HLA locus on 6p21.3. Exclusion of this locus abolishes the difference between NSTEMI and STEMI but not the association of the remaining SNP's with MI.
Our results add to the notion that common differences in gene expression influence the risk for developing MI. We show that the risk conferred by eQTL's is conditional on a clinically relevant classification of MI and identify a locus contributing to this difference.

P09.163
Identification of two SNPs located upstream and intron of CPT1B gene and HLA DQB1*0602 allele in Turkish patients of narcolepsy with and without cataplexy

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Narcolepsy is a chronic neurologic disorder characterized by excessive daytime sleepiness, cataplexy and clinical manifestations of abnormal REM sleep. Narcoleptic pathogenesis is triggered by genetic and environmental factors and strongly associated with HLA-DQB1*0602. In recent reports it was established that SNP rs5770917 is associated with susceptibility to narcolepsy and also in strong LD with rs5770911. This study aimed to investigate positivity of HLA-DQB1*0602 allele and detect the differences in allele frequencies of two SNPs among case-control groups.
This study includes 31 narcolepsy with cataplexy (n=19) and without cataplexy (n=12) patients of Neurology Departments in Turkey and 55 healty individuals as a control. Patients and controls were explored for presence of HLA-DQB1*0602 by PCR and screened by sequencing whether the SNP rs5770917 and rs5770911 are associated with narcolepsy. Significance was assessed by chi-square and Fisher's exact test.
The HLA-DQB1*0602 allele was identified in 13 (68.4%) of patients with cataplexy, in 3 (25%) of patients without cataplexy and in 7 (12,7%) of controls. It was significantly more frequent in narcolepsy patients than in controls (p<0.001) and narcolepsy with cataplexy patients than in narcolepsy without cataplexy patients (p<0.05). The comparison of allele frequencies of two SNPs between patient and control groups yielded any significant results.
Recently, HLA-DQB1*0602 allele positivity was significantly high although the rates of the allele was lower in our narcolepsy with/out

cataplexy patients than the results observed in different populations. For further studies, the interaction between SNPs and HLA-DQB1*0602 should be analyzed in a large Turkish patient group.

P09.164
Study of the natriuretic peptide precursor gene $(TTTC)_n$ microsatellite polymorphism and plasma BNP levels in pre-eclampsia

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Backgound: There is a variable tandem repeat (TTTC) polymorphism of the natriuretic peptide precursor B gene (NPPB), which shows association with essential hypertension. Our aim was to identify this polymorphism in samples of pre-eclamptic patients and healthy controls. We also compared the natriuretic peptide B (BNP) concentrations.

Methods: Blood samples were collected from healthy pregnant normotensive women (n=235) and patients having pre-eclampsia (n=220). DNA was isolated and fluorescent PCR and DNA fragment analysis was performed for the detection of (TTTC) repeats. The plasma BNP concentration was measured by fluorescence immunoassay method.

Results: We detected 12 different repeats on the NPPB gene. The overall distribution of alleles and genotypes was significantly different between the control and pre-eclamptic groups. The number of 10-repeat genotype carriers showed significantly lower frequency in pre-eclamptics than in the healthy pregnants (p=0.032). Adjusted odds ratio: 0.19 (95% CI: 0.04-0.87). Similarly 12-repeat genotype carriers (p=0.037), adjusted OR: 0.53 (95% CI: 0.29-0.96). Contrary, the 11-repeat genotype carrier frequency was significantly higher in the pre-eclamptic group (p<0.001, adjusted OR 2.91 (95% CI: 1.75-4.84)). The concentration of the BNP was 9.75 pg/mL in the healthy controls and 32.40 pg/mL in the pre-eclamptic group (p<0.0001). The 11/11 genotype carriers had significantly higher BNP levels in the pre-clamptic group.

Conclusions: The NPPB gene (TTTC) microsatellite polymorphism showed significant difference in the distribution of alleles and genotypes between healthy pregnants and pre-eclamptic patients in an ethnically homogeneous population. The concentration of the BNP was higher in pre-eclamptics, and it showed association with the genotypes.

P09.165***
Mutations of *VANGL2* gene in human Neural Tube Defects

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Animal models were proven to be instrumental in deciphering the complex causation of Neural Tube Defects (NTDs). Particularly, these models demonstrated an essential role for the planar cell polarity (PCP) pathway, also called the non-canonical Frizzled/Dishevelled pathway, in neurulation. PCP is the process by which cells become polarized within the plane of an epithelium. The *Loop-tail* (*Lp*) mouse, that develops craniorachischisis, has been shown to carry missense mutations in the PCP core gene, *Vangl2.* In vertebrates, *Vangl2* has another homolog called *Vangl1* that has similar biochemical functions based on protein similarity and expression data. Recently, we identified eight novel rare missense mutations in *VANGL1* that were associated with human NTDs. To assess the role of the human ortholog *VANGL2* in the complex etiology of NTDs in humans, we resequenced this gene in a large multi-ethnic cohort of 673 NTD patients. We identified six novel heterozygous missense mutations in seven patients, that could be pathogenic based on genetic and initial validation data. Four of these mutations, p.Arg135Trp, p.Arg177His, p. Leu242Val, p.Arg270His, were predicted to be damaging to protein function using bioinformatics' tools, and two others, p.Thr247Met and p.Arg482His, affect highly conserved residues across evolution. Five mutations were identified in patients affected with closed spinal NTDs, suggesting that *VANGL2* mutations may predispose to thys type of NTDs. Our findings strongly implicate *VANGL2* in the genetic causation of spinal NTDs in a subset of patients and provide additional evidence for a pathogenic role of PCP signaling in these malformations.

P09.166
Sex modulates the effect of *DRD2/ANKK1* polymorphisms in smoking

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Several publications, have addressed the relationship between smoking behavior and the *DRD2* gene, especially focusing on the Taq1A polymorphism (which in fact, belongs to a *DRD2* neighbor gene, named *ANKK1*). However, strong heterogeneity among studies has been reported by meta-analysis. Here we analyze the potential role of Taq1A and other three neighbor polymorphisms (rs6277, rs2283265 and rs2734849) in relation to smoking status. Our sample is composed of 463 adult patients with ADHD and 608 non-ADHD adults, totalizing 1071 subjects. Smoking is more common among patients with than without ADHD (43% vs. 18%, respectively; p<0001) but this is not influenced by sex. Logistic Regression analysis, controlling for ADHD status and sex, showed a significant interaction between Taq1A X gender on smoking status (p=0.016). A similar result was observed for the SNP rs6277 in exon 7 of *DRD2* (p=0.076 for interaction). Results of both polymorphisms are suggestive of opposite effects in men and women, with trends towards predisposition in men and protection in women. We did not observe any significant main effect or interaction of the other two polymorphisms studied. Therefore, our results support the hypothesis that the association of the Taq1A and smoking may be modulated by sex, which would help explain the heterogeneity in findings on the Taq1A/smoking association of the last several years. This also seems to be the case involving the rs6277 polymorphism, although further studies are needed to sustain this hypothesis.

P09.167
Polymorphisms in FGF1 gene are associated with nonsyndromic cleft lip and palate

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FGFs and their cell surface receptors (Fgfr) are a complex family of signalling molecules that play important roles in a variety of processes of embryogenesis and tissue homeostasis. The published data suggest that the FGF signalling pathway may contribute to as much as 3%-5% of non-syndromic CLP (NSCLP) and will be a consideration in the clinical management of CLP.

The aim of the study was to confirm the role of FGF1 gene in development of NSCLP.

Subjects and Methods: Thirty five SNPs in the FGF1 gene were genotyped using APEX-2 technology for allelic association with NSCLP. The data set consisted of 108 non-syndromic cleft lip and/or cleft palate patients samples and 182 unrelated non-affected and randomly selected individuals from Latvia as control population. Out of the 108 cleft cases, 79 had CLP and 29 had CL. Association analyses of cases and controls were performed using PLINK software version 1.07.

Results: Genotype distributions among study groups were in a Hardy-Weinberg equilibrium. We found very strong evidence that the FGF1 gene plays a significant role in the development of non-syndromic orofacial clefts. Three markers in the FGF1 gene were strongly associated with non-syndromic cleft lip and/or cleft palate (rs33992, p=0.001, OR=1.68, 95% CI=0.051-0.556; rs34010, p=0.0002, OR=0.48, 95% CI= 0.332-0.707; and rs2070715, p=0.025, OR=0.67, 95% CI= 0.465-0.951).

Conclusions: The results show that FGF1 gene contains genetic variations contributing to formation to clefts but additional studies should be made to clarify the possible involvement of these polymorphisms in the etiology of NSCLP.

P09.168
Association of NPBWR1 and NPBWR2 gene polymorphisms with hypertension.

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NPBWR1 and NPBWR2 are receptors of neuropeptide B (NPB) and neuropeptide W (NPW). This neuropeptide system is thought to have a role in regulating feeding behaviour, energy homeostasis, neuroendocrine function, however its role in humans remains unknown. The aim of the study was to characterize the genetic variance of NPBWR1 and NPBWR2 and investigate the possible correlation of identified polymorphisms with number of metabolic and cardiovascular traits and phenotypes. In total 6 polymorphisms were indentified in both gene loci from direct sequencing of 100 individuals. Genotype based association was performed in 1500 individuals from Genome Database of Latvian Population. Among 10 different traits included in this analysis hypertension was significantly associated with one missense SNPs from each coding part of NPBWR1 (rs33977775) and NPBWR2 (rs4809401). These SNPs and two SNPs from NPW gene were genotyped in additional group of 500 hypertensives and 500 validated controls. rs33977775 and rs4809401 were significantly associated with presence of hypertension (P=0.0018 and P=0.03 respectively). We have also identified significant epistasis between these SNPs (P=0.005). We also present functional importance of two identified NPBWR1 variants (Ile40Thr and Tyr135Phe) on cAMP inhibition in HEK-293 cells.

P09.169
Genetic polymorphisms in *PXR* associated with alcohol consumption and side effect of methadone maintenance treatment

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Objective: Methadone, a synthetic opioid, is well known as a maintenance drug to prevent heroin-addicted patients from experiencing withdrawal symptoms. In addition, nuclear receptor pregnane X receptor (*PXR*) is a xenobiotic nuclear receptor that binds ligands with different structures and is implicated in diverse functions. In this study, we tested whether *PXR* genetic polymorphisms are associated with alcohol consumption and side effect of methadone maintenance treatment.

Methods: A total of 366 heroin addicts undergoing methadone maintenance treatment were recruited in this study. Data were collected using interviewer-administered assessments, including Treatment Outcomes Profile (TOP), and Treatment Emergent Symptoms Scale (TESS). Twenty-five single-nucleotide polymorphisms (SNPs) of *PXR* were genotyped. After adjusting covariates, regression analyses were performed to identify individual association of *PXR* with side effect of methadone maintenance treatment, and joint association of *PXR* and *ALDH2* with alcohol consumption. Permutation-based approach was applied to correct for multiple testing.

Results: We found that 11 *PXR* SNPs were significantly associated with TESS, and 6 *PXR* SNPs were significantly associated with alcohol consumption after multiple testing correction ($P_{corrected}$ < 0.05). In addition, haplotype combination of "rs1523130-rs6785049-rs6438550" was significantly associated with average of alcohol consumption after multiple testing correction ($P_{corrected}$ < 0.05). Furthermore, We found rs6438550 of *PXR* was interacted with rs671 of *ALDH2* on alcohol use ($P < 10^{-4}$).

Conclusion: *PXR* genetic polymorphisms may have impact on the side effect of methadone treatment, moreover influence alcohol consumption through interacting with *ALDH2* gene in heroin addicts.

P09.170
Frequency of genotypes and rare alleles of genes implicated in lipid metabolism in children and adolescents according to family history of obesity.

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The aim of the study was to estimate the frequency of genotypes and rare alleles of gene polymorphisms 4a4b *eNOS*, I/D *ACE*, W64R *ADRB3*,Q/E27 and G/R16 *ADRB2*,G-75A and C+83T *ApoA1*,S19W and -1131T<C *ApoA5*,L162V *PPARA* in 132 children and adolescents aged 5 to 17. They were divided into 4 groups: (1) 72 individuals whose one or both of the parents suffered from abdominal obesity, (2) 19 individuals whose one or both parents had abdominal fat distribution without obesity, (3) 7 whose one or both parents had obesity but normal fat distribution, and (4) 34 whose parents did not suffer from obesity and had normal fat distribution. The criterion of obesity was body mass index > 27 kg/m^2. Abdominal fat distribution was assessed using waist/hip index, with upper limit of 0.9 for men and 0.85 for women. Gene polymorphism 4a4b *eNOS,* polymorphism of I/D *ACE* were detected by PCR. Polymorphisms G-75A and C+83T *ApoA1*, S19W and -1131T<C *Apo A5*,W64R *ADRB3*, Q/E27, G/R16 *ADRB2*,L162V *PPARA* were determined by PCR-RFLP method. We did not find any significant difference in frequency of genotypes and rare alleles of investigated gene polymorphisms between all studied groups. This might be explained by insufficient samples size. Further studies in larger samples using a different approach, namely a study of combination of genotypes and rare alleles might bring more conclusive results.

P09.171
A clinical and genetic study of childhood and adolescent obesity

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Obesity is determined by genetic, environmental and behavioral factors acting through the physiological mediators of energy intake and energy expenditure. The aim of this study was to define the most characteristic clinical, genetic, anthropometric and laboratory findings in childhood obesity in an attempt to find the most efficient way to diagnose a genetic cause of obesity. Subjects and Methods: All cases were subjected to full history taking, and full clinical examination including dysmorphology and pubertal assessment. Endocrinal causes were excluded. Serum leptin and insulin where estimated whenever a monogenic cause of obesity was suspected. Conventional and high resolution chromosomal analysis, FISH and molecular studies where done in selected cases. Results: This study included 30 obese children and adolescents. Among the 30 studied cases, 17 had syndromic obesity (56.67%), 7 had simple obesity (23.33%) and 6 had monogenic obesity (20%). Within the 6 diagnosed cases with monogenic obesity, we detect one novel missense mutation in the leptin LEP gene (N103K) and another novel nonsense mutation in the leptin LEP gene (W121X) as well as missense mutation in the Leptin receptor gene LEPR (P316T).

Conclusion: Although genetic causes of obesity are rare autosomal recessive disorders, high consanguinity rate in our society will lead to the discovery of a high number of monogenic and pleiotropic obesity. Early screening and regular follow up for obesity and its complications is particularly indicated for patients with syndromic forms of obesity, together with genetic counseling of the parents.

P09.172
Genotype x nutrient association of single-nucleotide polymorphisms in obesity-related genes with native dietary composition in the Central-European population

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As the personal food preferences can either enhance or suppress the development of obesity, we investigated native dietary composition as a specific trait related to obesity and we determined whether genetic variations in leptin (LEP), LEP receptor (LEPR), adiponectin (ADIPOQ), IL-6 and pro-opiomelanocortin (POMC) underlie specific native dietary composition as well as obesity-related anthropometric traits. The total of 520 individuals of Czech Caucasian origin were

enrolled into the present study and 7-d food records were obtained from the study subjects along with selected anthropometric measurements and podometers records. Independently of the BMI of the individuals, common variations in LEP and LEPR genes were associated with specific eating patterns, mainly with respect to timing of eating. The LEP +19A/G polymorphism served as an independent predictor for BMI, percentage of body fat and skinfold thickness and significantly affected the time structure of the daily energy intake. The POMC Rsa I polymorphism was associated with percentage of body fat. The ADIPOQ +45T/G polymorphism was associated with the thickness of the subscapular skinfold. The LEPR Gln223Arg polymorphism was associated with multiple parameters, including diastolic blood pressure, meal sizes during the day and plasma ADIPOQ levels. In a separate sub-analysis, soluble leptin receptor (sObR) plasma levels and LEP:sObR ratio were significantly correlated with systolic blood pressure(beta = - 0.66, P = 0.002; beta = -1.23, P = 0.02). To conclude, we report common allelic variants associated with specific feeding behaviour, obesity-related anthropometric traits and time structure of food intake.

P09.173
Investigation of genome wide association signals for obesity: synthetic association and haplotype analyses at the melanocortin 4 receptor gene locus

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Background: Independent genome-wide association studies (GWAS) showed an obesogenic effect of two single nucleotide polymorphisms (SNPs) more than 150 kb downstream of the melanocortin 4 receptor gene (MC4R). The SNPs might directly influence MC4R function/expression, or they are on a haplotype that predisposes to obesity or includes functionally relevant genetic variation (synthetic association). MC4R is an ideal model to explore synthetic association.

Methodology/Principal Findings: We analyzed a genomic region (364.9 kb) encompassing the MC4R in GWAS data of 424 obesity trios (extremely obese child/adolescent and both parents). SNP rs12970134 showed the lowest p-value (p = 0.004); conditional analyses on this SNP revealed that 7 of 78 analyzed SNPs provided independent signals. These 8 SNPs were used to derive two-marker haplotypes. The confirmed (363 independent obesity trios) obesity effect haplotype comprises the MC4R. Including MC4R coding variants in a joint model had almost no impact on the effect size estimators expected under synthetic association. Haplotype analyses were also conducted for the 31 additional GWAS-derived SNPs; 2 haplotypes revealed better results than the single SNPs, confirmation is ongoing.

Conclusions/Significance: A haplotype reaching from 5' of the MC4R to at least 150 kb from the 3' end of the gene showed a stronger association to obesity than single SNPs. Synthetic association analyses revealed that MC4R coding variants had almost no impact on the association signal. Our data underscore the problems underlying the identification of relevant mutations depicted by GWAS derived SNPs (Scherag et al., PLoS One 2010).

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P09.174
Orofacial clefts and MTHFR gene polymorphisms in Slovak population

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Nonsyndromic orofacial clefts are multifactorial birth defects with folate deficiency suspected as one of the risk factors. We evaluated two frequent polymorphisms of MTHFR gene C677T and A1298C in 65 Slovak patients with nonsyndromic orofacial clefts (OFC) (38 males, 27 females); 53 of all had cleft lip or cleft lip and palate (CLP) and 12 had isolated cleft palate (CP). The control group consisted of 290 healthy newborns (147 males, 143 females). The results revealed significantly higher proportion of T allele in C677T polymorphism in the whole OFC patients´ group (p=0.043) as well as in the CLP group 0.037) compared to the controls. Genotype analysis, however, did not show significant prevalence of mutant genotypes (OFC p=0.398, CLP p=0.697), only of heterozygotes (OFC p=0.014, CLP p=0.002). Analysis of the groups according to sex revealed again significant abundance of C677T heterozygotes, but not TT mutants in females. The results in the male groups did not show any significant differences compared to the controls in C677T polymorphism. The analysis of the A1298C polymorphism did not reveal any significant differences between the patients and controls, in the whole OFC group as well as in the CLP group and according to the sex. We can conclude that the two common polymorphisms of MTHFR gene did not pose the risk of OFC in our patients´ group from Slovakia; anyhow, we are aware of a need of a larger patients` sample and continue in the patients´ recruitment.

P09.175
Genome-wide association study reveals new candidate loci for hand osteoarthritis

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Objective. Osteoarthritis (OA) is a complex disease common in the elderly. Our aim was to study single nucleotide polymorphisms (SNPs) using a genome-wide association (GWA) data in a setting of hand OA cases versus healthy controls.

Methods. The study subjects were over 56 years of age and were part of Finnish Helsinki Birth Cohort Study sample genotyped using Illumina HumanHap 610 GWA chip. Hand joints of the study subjects were visually evaluated and individuals with Heberden's nodes in at least one DIP joint were graded as affected (n=524). Individuals graded as healthy (n=970) had visually healthy symptomless finger joints. Association with individual SNPs was monitored using the Plink program. Age, sex and two principal components differed between the cases and controls and were used as covariates.

Results. In our hand OA GWA analysis the most significant results were for SNP flanking the CD28 gene in 2q33 ($p=4.37x10^{-6}$, OR=1.68, 95% CI 1.35-2.10); SNP in the SUCLG2 gene in 3p14 ($p=6.15x10^{-6}$, OR=0.62, 95% CI 0.50-0.76) and SNP in the SLC22A3 gene flanking lipoprotein genes LPAL2 and LPA in 6q25 ($p=1.61x10^{-5}$, OR=1.41, 95% CI 1.20-1.64).

Conclusion. Variants in regions harbouring CD28, SUCLG2, SLC22A3, LPAL2 and LPA genes showed suggestive evidence for association with hand OA. CD28 has been shown to affect T-cell functions and IL2 production. CD28 and LPA genes have been shown to play role in rheumatoid arthritis. Results did not reach genome-wide significance in the initial screening phase but replication effort in a large study set is ongoing.

P09.176
Genome-wide association study identified TIMP2 genetic variant with susceptibility to osteoarthritis

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Osteoarthritis (OA) is the most common degenerative joint disorder in the elderly population. To identify OA-associated genetic variants and candidate genes, we conducted a genome-wide association study (GWAS). A total 3,793 samples from a community-based

epidemiological study were genotyped using the Affymetrix SNP 5.0. An intronic SNP (rs4789934) in the TIMP2 (tissue inhibitor of metalloproteinase-2) showed the most significance with OA (odd ration [OR] = 2.06, 95% confidence interval [CI] = 1.52-2.81, p = 4.01×10^{-6}). Furthermore, a polymorphism (rs1352677) in the NKAIN2 (Na^+/K^+ transporting ATPase interacting 2) was suggestively associated with OA (OR = 1.43, CI = 1.22-1.66, p = 7.01×10^{-6}). The present study provides new insights into the identification of genetic predisposing factors for the prevention of intractable pain from OA.

P09.177**

1,000 genomes based imputation discovers novel locus for osteoarthritis

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The genetic architecture of osteoarthritis (OA) has not been well characterized yet; to date only two loci have been reproducibly associated with knee OA in Europeans at genome-wide significance levels. As part of the arcOGEN consortium, we have carried out a genome-wide association study (GWAS) in 3,177 cases (knee and/or hip OA) and 4,894 UK controls. We performed genome-wide imputation using the August 2009 release of the 1,000 genomes project and tested for association with OA. We identified 7 loci with p<7E-06 and genotyped them in an independent set of 5,165 arcOGEN cases and 6,155 UK controls. rs11842874 in intron 3 of *MCF2L*, the guanine nucleotide exchange factor DBS, on chr13 showed evidence for replication (p=2.6E-03, MAF ~7%). Based on 8,066 Stage 1 individuals with imputation and directly typed data, there were 24 discordant alleles (overall allelic concordance 99.85%; minor allele concordance 97.93%). Replication was further sought in an independent set of 1,686 knee and/or hip OA cases and 743 UK controls, and in 207 knee and/or hip OA cases and 2,618 controls from Estonia. The fixed-effects meta-analysis odds ratio of the discovery and replication datasets for the major allele A was 1.23 [95%CI 1.14-1.33], p=4.33E-08 (10,235 cases, 14,410 controls in total).

P09.178

The association between serum osteopontin levels and OPN Ala250 polymorphism in urolithiasis

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Background: Osteopontin (OPN) is one of the urinary proteins having an important role in stone formation.Recently,OPN Ala250 (rs1126616) polymorphism along with other OPN SNPs have been investigated in order to define their role in urolithiasis.The aim of our study was to investigate OPN rs1126616 polymorphism and serum OPN levels in 106 urolithiasis patients together with 88 healthy controls.

Material and Methods: Stones were analyzed for their chemical composition by using X-Ray diffraction method.The study groups were genotyped by PCR-RFLP and serum OPN levels were measured by ELISA.

Results:OPN genotype frequencies in urolithiasis patients and control group were shown in Table1. In urolithiasis patients, OPN Ala250 heterozygote and homozygote carriers were almost 2 fold higher compared to controls (p=0.001). Serum OPN levels were higher in control group than urolithiasis patients (p=0.000) (Table1).

Conclusion: Ala250 polymorphism was significantly higher in Turkish urolithiasis patients by contrast their serum OPN levels were significantly lower.These results might be indicating the important predictive role of serum OPN and Ala250 polymorphism in urolithiasis patients.

	Urolithiasis Patients n=106	Healthy Controls n=88	*P* value
Age	43,3±13,3	38,4±14,9	*0,147*
Sex (F/M)	31/75	34/54	*0,197*
CC (%)	51,9	76,1	***0,000***
CT (%)	32,1	8	
TT (%)	16	15,9	
CT+TT (%)	48,1	23,9	***0,001***
Serum OPN level (ng/ml)	4,4±1,2	9,5±2,2	***0,000***

P09.179

Lack of associations of CALCR and LCT gene polymorphisms with bone mineral density and osteoporotic fractures in the Volga-Ural region of Russia

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Determination of bone mineral density (BMD) and formation of osteoporotic fractures are multifactorial and under complex genetic and environmental regulation. Calcitonin receptors (*CALCR*) participate in calcium metabolism and polymorphisms *CALCR* gene contribute to the variation of BMD and underlie osteoporotic fractures. Polymorphisms of LCT gene can lead to indigestion disorders and as a consequence, to disturbance of calcium absorption and reduce of BMD level. We examined associations of polymorphisms c.1377C>T (rs1801197) in the *CALCR* gene and -13910C>T (rs4988235) in the *LCT* gene with fractures and level of BMD in 251 Russian and Tatar women with postmenopausal osteoporosis and 100 Russian men with osteoporotic fractures and matched control (n=399). In Tatar and Russian women with postmenopausal osteoporosis *CALCR*T* allele frequency distribution was lower than in control groups (0.50 and 0.704, respectively), in Russian men with osteoporosis *CALCR*T* allele frequency was higher (0.655) compared with the control group (0.60). The frequency of genotype *LCT*C*C*, determining hypolactasia, was 51.61% in Russian women with fractures and 40% in control group. In this case, *LCT*C*C* genotype frequency in Tatar women of control group (63%) was higher compared with group of patients (45.5%), suggesting the existence of other genetic variants determining the synthesis of lactose in Tatars. Thus, the study of polymorphic loci c.1377C>T in the *CALCR* and -13910C>T in the LCT gens revealed no association of studied loci with fractures development and bone mineral density levels in women with postmenopausal osteoporosis of Russian and Tatar ethnic origin, as well as in Russian men with osteoporosis.

P09.180

Association of VDR, COL1A1, CALCR and BGLAP polymorphisms with susceptibility to steroid osteoporosis in patients with idiopathic pulmonary fibrosis (IPF)

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Background. Osteoporosis, as complication of glucocorticosteroids (GCS) treatment, is a serious medical and economic problem. Osteoporosis is a polygenic disorder.

Aim. To assess effectiveness of steroid osteoporosis prevention by antiresorptive agents (ARA - calcium, vitamin D, bisphosphonates, calcitonin) in patients with IPF with different genetic predisposition to osteoporosis.

Subjects. 84 Caucasian patients with IPF, 16 men and 68 women, age 56.5±10.9 years, treated with GCS and ARA.

Methods. Bone mineral density (BMD) measuring by DEXA, patients' questionnaires and genotyping approaches were used. We investigated 5 SNPs by PCR-RFLP analysis: 2 sites in vitamin D receptor gene (VDR-BsmI and VDR-FokI), rs1801197 in calcitonin receptor gene, rs1800012 in collagen type1 alpha1 gene and HindIII-polymorphism in osteocalcin gene.

Results. Frequencies of minor alleles were 0.375 (VDR-BsmI), 0.411 (VDR-FokI), 0.185 (COL1A1), 0.280 (CALCR), 0.179 (BGLAP). Associations between VDR-FokI and BGLAP genotypes and BMD

(p=0.02 and p=0.05, respectively), between GCS doses and bone fractures occurrence (p=0.01) were found. Multiple regression analysis showed significant influence of polymorphisms VDR-FokI, COL1A1, CALCR and BGLAP on BMD (p<0.01) but only minor fraction of susceptibility can be explained by these polymorphisms (adjusted R^2=0.238). Environmental factors, firstly ARA intake, seems to have stronger influence on BMD and bone fractures occurrence than genetic predisposition.
Conclusion. ARA administration is an essential way to prevent and treat steroid osteoporosis in patients with IPF, including individuals with genotypes associated with increased risk of osteoporosis progression. Genetic analysis is recommended to reveal subjects with increased risk of osteoporosis.

P09.181
Otitis media prone children: Association with cytokine gene polymorphisms and environmental risk factors

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Otitis media (OM) prone status in children results from complex interactions between genetic factors of the host, exposure to pathogens and environmental risk factors. Cytokine gene polymorphisms, determining the response to infection, may play an important role in multifactorial etiology of this frequent childhood disease. The objective of this study was to investigate the role of specific cytokines gene polymorphisms in OM prone children, as well as significance of association of cytokine gene polymorphisms and environmental risk factors (frequent respiratory infections, passive smoke exposure, day-care attendance and breast feeding practice) in children with recurrent episodes of otitis media. A total of 132 (OM prone and controls), both sex children were enrolled in the study. Single nucleotide polymorphism of IL2, IL6, IL10 and TNFα genes (TaqMan predesigned Genotyping SNP assay) and presence of environmental risk factors, were studied for all children. Results: Explicit bimodal distribution of OM susceptibility appearance, indicate that early childhood and preschool age plays important role in development of OM prone status. Out of 5 examined gene polymorphisms involved in regulation of inflammatory response, significant contribution to genetic predisposition to OM in Montenegro children was found for $IL10_{-1082}$ (G→A) gene. Conclusion: Our study has clearly pointed the significance of $IL10_{-1082}$ gene polymorphism in complex multifactorial pathogenesis of OM susceptibility in Montenegrin children, especially in interaction with environmental risk factors.

P09.182
The glutathione peroxidase 1 (*GPX1*) single nucleotide polymorphism Pro198Leu: association with life span and coronary artery disease in Russian population

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According to the free radical theory of aging, accumulation of oxidative damages determines life span. Also it is suggested that oxidative stress contribute to the development of various diseases including coronary artery disease (CAD). Inactivation of reactive oxygen species is performed by antioxidative enzymes. In this study we genotyped 5 SNPs in 4 genes for antioxidative enzymes (*CAT, SOD2, PON1, GPX1*) in four groups: patients with CAD (n=172), long-livers - above 90 years (n=210), people with early death (before 55 years) from cardiovascular diseases (n=94) and Russian population as control group (n=412). In particular, polymorphism in *GPX1* Pro198Leu (rs 1050450) has shown differences between the groups. The genotype distribution in control group was in Hardy-Weinberg equilibrium. We have found significant higher allele *T* frequency in men with CAD - 34.84% (χ^2=5.228, p=0.022; OR=1.46) and in men with early death from cardiovascular diseases - 38.16% (χ^2=6.461, p=0.011; OR=1.69) compared with control men - 26.8%. Moreover, significantly higher genotype *TT* frequency has been shown in patients with CAD and myocardial infarction (MI) before age 50 - 19.44% compared with control group - 7.28% (χ^2=9.55, p=0.002). The *TT* frequency in long-livers (4.39%) was the lowest and significantly different from CAD group - 12.79% (χ^2=8.07, p=0.0045) and from CAD subgroup with MI before 50 - 19.44% (χ^2=14.49, p=0.0001). Thus our results indicate that allele *T* (Leu) of *GPX1* Leu198Pro polymorphism is unfavorable for successful ageing. It predisposes to coronary heart disease, earlier myocardial infarction (before age 50) and earlier death (before age 55).

P09.183
Paraoxonase 1 gene (PON1) polymorphisms are associated with abdominal aortic aneurysms (AAA) in Polish population

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Abdominal aortic aneurysm (AAA) is an age-related vascular disease and an important cause of morbidity and mortality in developed countries. The pathogenesis of AAA is multifactorial with a strong genetic component. Previous studies show that polymorphisms in genes involved in homocysteine metabolism may contribute to risk for AAA developing. However up to now there are very few appropriately powered association studies considered the role of specific genetic variants.
We determined relationship between three functional polymorphisms in paraoxonase 1 (PON1) gene: -108C>T (rs705379), L55M (162T>A; rs854560), Q192R (575A>G, rs662) and AAA occurrence in Poland. PON1 is an antioxidant enzyme, which detoxify homocysteine thiolactone in vascular vessels. The study was conducted in groups of 525 AAA cases, 425 controls and in random population sample of 220 subjects. Polymorphisms were studied by PCR-RFLP method. Haploview was used for haplotype analysis.
Coding region polymorphisms L55M and Q192R were in strong linkage disequilibrium (D'=0,76), which results in relatively low frequency of 55M-192R haplotype in Polish population (0,023). The frequency of this rare haplotype in patients (0,008) was significantly lower as compared to that in controls (0,018, p=0,04). The 5,3-fold higher risk of AAA was noted in homozygotes of 55L-192R haplotype (subjects with -108CT/55LL/192RR genotype; p=0,003). The distribution of 55-192 haplotypes in Polish cohort of AAA patients corresponds to that observed by Giusti et all (2008) in Italian population.
In conclusion, our results confirm observations that functional variants of PON1 gene influence AAA risk.
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P09.184
Analysis of mitochondrial haplogroups in patients with Parkinson´s disease in Slovak republic

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Parkinson disease is the second most often neurodegenerative progressive dissease with prevalence 1 % in 65 years old persons. It is caused by selective degradation of dopaminergic neurons in substantia nigra pars compacta. Etiology of Parkinson disease can be explained by number of factors, including effect of nuclear genes and mitochondrial DNA variation. The first evidence that mitochondrial polymorphisms could be implicated in etiology of PD came from study of T4366C associated with increased risk of Parkinson disease in Europe. Abnormality of function of mitochondrial electron transfer chain has been reported in many PD cases. Beside haplogroup determining SNPs, may mitochondrial haplotypes and haplogroups share polymorphisms modulating ,,coupling efficiency" of mitochondrion with protective or pathological effect in PD expression. Especially variation in tRNA, 16S RNA and genes for cytochrome b in mtDNA have been identified like possible modulation factors in pathogenesisof PD.
We genotyped 199 PD pacient and 382 control subject and detect

decreased frequencies of haplogroup U (OR= 0, 55; 95% CI: 0,39- 0,90), its subhaplogroup U4 (OR= 0, 26; 95% CI: 0,07- 0,89) and cluster UK (OR= 0,60; 95 % CI: 0,39- 0,92). Decreased frequencies of UK, K + U5b + U4 + J and K + U5a + U4 + J we reported only between men.We also detected increased frequency of T1 (OR= 8,00; 95% CI: 1, 68- 38, 04) in slovak PD pacients. Its protective or possible pathogenic effect of this haplogroup has not been described so far.

P09.185
Stable elevation of FAS mRNA level in peripheral blood lymphocytes of patients with LRRK2-associated Parkinson's disease over time

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Mutations in the Leucine Reach Repeat Kinase 2 (LRRK2) gene are the most frequent cause of familial Parkinson's disease (PD). Although the precise physiological and pathological role of LRRK2 is unclear, direct link between mutant LRRK2 and apoptosis has been suggested. Earlier we found the increased FAS mRNA level in patients with LRRK2-associated PD compared to controls (persons without neurological disorders). The aim of our present work was to examine the same patients with LRRK2-associated PD over the three-year period and evaluate FAS mRNA level in peripheral blood lymphocytes (PBL) after 1h, 24h, 48h of incubation (37°C, 5% CO2). FAS mRNA levels were estimated in PBL of four patients with LRRK2-associated PD (n=3 G2019S; n=1 V1613A, mean age 65±10) and of nine controls (mean age 61±7). At 1h, 24h, 48h PBLs were harvested, total mRNA extracted and cDNA synthesized. FAS mRNA levels were estimated by means of quantitative real-time PCR with TagMan probes. The level of G protein (GNB2L1) mRNA was used as internal control. At 24h mRNA levels were slightly increased compared to 1h in both groups but this difference was not statistically significant. FAS mRNA level was higher in patients with LRRK2-associated PD compared to controls at 1h ($p<0.03$) and at 24h ($p<0.05$). Our results suggest LRRK2 mutations may lead to the activation of FAS expression in PBL of patients with LRRK2-associated PD and this induction in stable over time.

P09.186
Analysis of exon rearrangements in PARK2, PINK1 and SNCA genes in patients with Parkinson's disease from Russia

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To date, a number of genes involved in the pathogenesis of Parkinson's disease (PD) have been identified. We analyzed rearrangements in exons 1-12 of the PARK2 gene in 170 sporadic early-onset (EOPD, age at onset < 45) and 183 sporadic late-onset (LOPD, age at onset > 45) patients with PD. Exon 7 rearrangements in PINK1 gene were studied in a mixed group of 214 patients with familial and early-onset sporadic PD. Rearrangements in exons 4-6 of the SNCA gene were studied in 61 patients with autosomal dominant PD. All the patients were from Russia. The frequency of EOPD and LOPD patients carrying these mutations in PARK2 gene was 12.4 and 3.8%, respectively. The most frequent rearrangements were detected in exons 3 and 4. The odds ratio for EOPD in individuals carrying PARK2 gene exon deletions and duplications was 3.54 (0.95% CI, 1.465-8.569; P = 0.006). We also found a correlation between exon rearrangements in PARK2 gene and some clinical features of PD. Exon 7 deletion in PINK1 gene was found in 5 patients. The analysis of the SNCA gene revealed no increase in the exon dosage. The results of our study let us conclude that exon rearrangements in PARK2 gene have a significant role in the pathogenesis of sporadic PD in patients from Russia. The role of exon deletions in PINK1 gene remains to be elucidated and multiplications of the SNCA gene play insignificant part in the pathogenesis of autosomal dominant PD in Russia.

P09.187
Androgen levels and metabolic parameters are associated with a genetic variant of F13A1 in women with PCOS

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Polycystic ovary syndrome (PCOS) affects about 6-15 % of all women and is therefore the most common hormonal disorder among women of reproductive age. PCOS is associated with a variety of clinical problems like infertility, obesity and insulin resistance. This study aimed to evaluate allelic F13A1 variants - a blood coagulation factor gene - for its influence in PCOS patients.

We investigated the effect of an intronic SNP (A/G) in the F13A1 gene in 371 PCOS patients and 133 normal controls. Metabolic and hormonal measurements, oral glucose tolerance tests (oGTT) and a specialised hirsutism score (modified Ferriman-Gallwey method) were determined.

Genotype frequencies of the F13A1 SNP did not deviate from Hardy Weinberg equilibrium and were equivalent in PCOS and controls. In the dominant genotype model, in all PCOS patients and especially in overweight/obese PCOS patients (BMI ≥ 25), the G allele of F13A1 was associated with significantly higher levels of free testosterone and DHEAS as well as decreased levels of SHBG in the overweight/obese group, which was only seen as a trend in all patients. The G allele was additionally associated with insulin metabolism in lean PCOS patients (BMI < 25) since the AUC of insulin was significantly lower compared to the A allele.

We demonstrate an association of F13A1 gene variants with androgen levels in PCOS patients with increased BMI as well as metabolic parameters in lean patients. This might be of high importance for diagnostic and therapeutic aspects in this frequent disease.

P09.188
Pharmacogenetic study of adalimumab treatment in Crohn disease patients

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Crohn disease (CD) is one of the two major subtypes of Inflammatory bowel diseases and is characterized with chronic inflammation in whole gastrointestinal tract. Recently biological therapy using, himeric (infliximab (Remicade) or fully humanized (adalimumab (Humira) monoclonal antibodies against TNF-α are used in CD patients not responding to standard treatment or developing adverse drug effects. We have conducted prospective pharmacogenetic study in Slovenian refractory CD patients enrolled in adalimumab treatment. We used IBDQ and CDAI index to monitor therapy response. We have collected blood samples for DNA, RNA and protein isolation and analysis of some biochemical and immunological parameters (CRP) before treatment and in 4, 12, 20 and 30 weeks after first treatment with adalimumab. We have genotyped single nucleotide polymorphisms (SNPs) in selected apoptotic genes (CASP9, FCGR3A, FAS in TIMP-1), previously associated with response to infliximab. We found correlation between SNP in gene CASP-9 and response to treatment with adalimumab. Patients with C/C genotype had worse response after fourth weeks (p = 0,05) and 20 weeks (p = 0,031) of treatment compared to patients with C/T and T/T genotype. Results of our study are comparable with previous study where patients were treated with infliximab and patients with C/C genotype in CASP-9 gene had worse response to therapy. We also found correlation between polymorphisms in genes CASP-9 (SNP rs4645983) and FCGR3A (SNP rs396991) in Slovenian patients with CD involved in this study. Our results provide a step forward to identification of efficient pharmacogenomic biomarkers for individualized therapy in CD patients.

P09.189
Exome sequencing identifies a set of genes that may be associated with escitalopram treatment response in major depression.

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Antidepressants are widely used in the treatment of major depression although their efficacy is unsatisfactory - approximately 30% of patients do not fully recover even after several treatment trials. Interindividual genetic differences may contribute to the variability in antidepressant response. Association studies applying the candidate-gene strategy to identify genetic markers influencing antidepressant response in major depression have produced some results, many of which are inconsistent or not transferable to other study samples.

We report on the first application of full exome sequencing to antidepressant pharmacogenetics. From a sample of 126 individuals diagnosed with major depression and treated with escitalopram over 12 weeks, we selected 5 extreme responders and 5 extreme non-responders based on their phenotype - responders were patients who showed good treatment response after 10 mg of escitalopram, non-responders were resistant to treatment even after raising the dose to 20 mg daily. Complete exome sequencing of these patients was performed at the Beijing Genomics Institute using Illumina Genome Analyzer IIx platform.

By comparing the allele counts of previously known SNPs and novel polymorphisms in five responders and five non-responders, we identified a set of genes that may be associated with the difference in response to escitalopram treatment. The most promising findings have been replicated in the remaining 116 patient samples, and in the GENDEP sample which consists of 394 patients treated with escitalopram.

P09.190
Genome wide association analysis of rheumatoid arthritis patients treated with anti-TNF medication. Results of the DREAM registry

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Background: Treatment strategies blocking tumor necrosis factor (anti-TNF) have proven very successful in patients with rheumatoid arthritis (RA). However, a significant subset of patients does not respond for reasons that are unknown, and there is currently no means of identifying these patients.

Objective: We aimed to identify genetic factors predicting anti-TNF treatment outcome in patient with RA using a genome-wide association approach.

Methods: We selected RA patients treated with anti-TNF agents from the Dutch Rheumatoid Arthritis Monitoring (DREAM) registry. Disease activity scores (DAS28) at baseline and after 14 weeks was available of 508 patients. Single nucleotide polymorphisms (SNPs) markers were genotyped using the Illumina HumanHap550-Duo BeadChip or the Human660W-Quad. Association analysis using the relative DAS28 change as outcome was performed using the whole-genome association analysis toolset PLINK.

Results: 511.499 SNPs and 502 patients passed quality control. 75 SNPs showed suggestive association (uncorrected p-value<10^{-4}) with relative DAS change. Candidate genes, among the top ten associated SNPs, that can be linked to anti-TNF or RA are *ASPH* (p=1.36*10^{-6}), a gene involved in calcium homeostasis and regulated by *STAT4*, one of the confirmed risk factors for RA and the genes *TIAM1* (p=2.79*10^{-6}) and *UBE2E2* (p=1.21*10^{-5}), both play a role in cell death, one of the working mechanisms of anti-TNF therapy.

Conclusions: The identified genes may serve as new biomarkers predicting anti-TNF response. However, significant findings need to be replicated in other patient cohorts. Confirmed biomarkers can be used to personalize medication for the individual patient.

P09.191
Assessment of a pharmacogenomic marker panel in a clinical biorepository

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Genetic variants associated with adverse events or treatment efficacy are often rare (less than 5% minor allele frequency) or located in difficult-to-assay regions of the genome. Genotyping arrays used for genome-wide association (GWA) studies, which are designed to capture most of the common variation in the human genome, may not be adequate for pharmacogenomics studies. To address this, Illumina has designed the absorption, distribution, metabolism, and excretion (ADME) Core Panel, a fixed content panel of 184 pharmacogenomic-related markers in 34 genes. Because these variants are rare and difficult to assay, there is a lack of reference data in the literature or reference populations such as the International HapMap Project. There is also little data on how well the ADME Core Panel performs.

As a pilot study, we genotyped 320 European Americans prescribed 5 or more unique drugs from BioVU, a de-identified clinical population from Vanderbilt University, to see if certain pharmacogenomic variants may be unequally distributed in patients who received many medications. We also genotyped HapMap samples as well. We abstracted allele frequencies from public repositories as well as the literature for markers targeted by ADME. Preliminary results suggest that 30% of ADME targeted markers do not have reference allele frequency data in HapMap or the literature. Of the 320 samples genotyped on ADME, 98.8% of markers had call rates >95% and HWE p>0.0001. Allele and genotype frequencies differed between reference data and the clinical population for 3 markers (p< 10-5), warranting a follow-up study to explain these results.

P09.192
Genetic and environmental factors influencing the Placental Growth Factor (PlGF) variation in two populations.

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Placenta Growth Factor (PlGF), a member of the VEGF family, is a key molecule in angiogenesis and endothelial cell growth and migration. Several studies have been performed to correlate PlGF plasma levels with different pathological conditions (i.e.: pre-eclampsia, inflammation, prognosis of various types of cancers) but, to date, no information is available regarding the genetic basis of PlGF variability. Furthermore, even if the effect of environmental factors (i.e. cigarette smoking) on angiogenesis has been explored, no data on the effect of smoking on the PlGF have been reported so far. We investigated the PlGF variability in two cohorts: a study sample from two isolated villages in Cilento region, South Italy (n=1251) and a replication sample from general Danish population (n=2647). A significant difference in the PlGF mean levels was found between the two populations. However, in both samples, we observed a strong correlation of PlGF levels with ageing and gender, men displaying PlGF levels significantly higher than women. Interestingly, smoking was also found to influence the trait in the two populations, although differently. The association between five single nucleotide polymorphisms (SNPs) located in the PlGF gene and the levels of the protein in the plasma was investigated. We observed a significant association between two polymorphisms and PlGF plasma levels (p<10-2) in the Cilento sample and this association was replicated in the Danish sample (p<10-9). These results, for the first time, support the hypothesis of the presence of genetic and

environmental factors influencing PlGF plasma variability.

P09.193
Association of genetic polymorphisms degradome pathway genes with children chronic lung disease susceptibility

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Proteases a large group of enzymes involved in multiple physiological and pathological processes in lung tissue. Through protein degradation and turnover proteases play an essential role in lung tissue remodeling and repair during the inflammatory response and may be important in the development of chronic lung disease. In our case-control study we investigated role of genetic risk factors to susceptibility of chronic lung disease in children.

The genotypes of MMP1 (-1607G/GG), MMP2 (-735C/T), MMP9 (-1562C/T), MMP12 (-82A/G) genes in 235 patients with chronic respiratory diseases and 323 healthy children were detected by the PCR-RFLP method.

We observed significantly increased susceptibility to chronic lung disease in children for the MMP12 (-82A/G) gene compared with healthy children (x2=5.85, P=0.016). The allele A of was identified as a risk allele for chronic lung disease in children (OR=1,93, 95%CI 1.10-3.40). Whereas the allele G was more frequent in healthy children, and was identified as a protective allele (OR=0,52, 95%CI 0,29-0,91). The A(-82) MMP12 - GG(-1607) MMP1 haplotype was also significantly associated with increased susceptibility to chronic lung disease in both groups (OR=1,40, 95%CI 1,09-1,81). The genotype and allele frequencies of MMP1 (-1607G/GG), MMP2 (-735C/T), MMP9 (-1562C/T) genes do not significantly differ in groups.

The results of the study suggest the genetic polymorphisms in degradome pathway genes may play a significant role in the development of chronic lung disease in children.

P09.194
Comparative study of allele frequencies of PAI-1, MTHFR and FV gene polymorphisms in children with Henoch-Schönlein purpura, Schamberg Disease and in healthy children.

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Objectives: Comparative analysis of alleles and genotypes distribution of plasminogen activator inhibitor 1 (PAI-1) -675 4G/5G insertion-deletion polymorphism, methylenetetrahydrofolate reductase (MTHFR)- 677C/T polymorphism and blood coagulation factor V (FV) 1691G/A polymorphism in (1) children with Henoch-Schönlein purpura (HSP), (2) in children with Schamberg Disease (SD) and (3) in healthy children. Patients and methods. HSP group consisted of 40 patients (20 boys and 20 girls), SD-group consisted of 32 patients (15 boys and 17 girls). Control group was composed of 31 apparently healthy children (20 boys and 11 girls). PAI-1-675 4G/5G insertion-deletion polymorphism was analysed by PCR method, MTHFR 677C/T and FV 1691G/A polymorphisms were analysed by PCR-RFLP method. Statistical significance of differences between groups was assessed using χ2 tests. Results. There was no statistically significant (p>0.05) difference in allele and genotype distribution of analysed polymorphisms between HSP group, SD group and control group. The genotype distribution was in accordance with Hardy-Weinberg equilibrium for all variants. Genotype 5G/5G PAI-1-675 was not found in SD group. 1691A allele of the FV was not found in any group (all children has “normal” GG genotype FV 1691). Conclusion. In this study we did not find PAI-1-675 5G/5G genotype in children with SD and no A -allele FV 1691G/A in any of studied groups.

P09.195
Analysis of polymorphism of COL1A1 and VDR genes in children with congenital scoliosis

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Vitamin D receptor gene (*VDR*) and the gene encoding alpha chain of collagen type 1 (*COL1A1*) are candidate genes in the development of congenital malformations of the skeleton.

OBJECTIVE: to study the distribution of alleles and genotypes of site Sp1 polymorphism of the *COL1A1* and of TaqI site polymorphism of the *VDR* genes in children with congenital scoliosis.

Patients and methods: the study group included 197 children aged 6 mo to 16 yo with various types of malformations of the spine. Control group included 60 children aged 2 to 17 yo without orthopedic pathology.

Molecular genetic study was carried out using PCR followed by restriction analysis.

RESULTS: Alleles and genotypes frequencies of Sp1 site of *COL1A1* gene and TaqI of *VDR* gene in children with congenital scoliosis and in controls are presented in Table.

Genotypes	Children with congenital scoliosis		The control group		P - value
	N	%	N	%	
COL1A1 Sp1					
SS	131	66,5	51	86,0	0,022
Ss	59	30	8	13,4	
ss	7	3,5	1	0,6	
allele S	321	81,5	110	92,4	0,007
allele s	73	18,5	9	7,6	
TaqI					
TT	78	39,6	39	65,0	0,002
TC	91	46,1	18	30,0	
CC	28	14,3	3	5,0	
allele T	247	62,6	96	78,3	<0,001
allele C	147	37,4	24	21,7	

CONCLUSION: The frequency of the *COL1A1* Ss genotype and s allele were significantly higher in patients with congenital scoliosis than in controls.

There were significant increase in the CC genotype frequency and C allele of *VDR* gene in children with congenital scoliosis compared with control subjects.

P09.196
A coding variant in NLRP1 is associated with autoimmune Addison`s disease

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Variations in the NLRP1 (previously, NALP1) gene have recently been reported to confer risk for vitiligo and associated autoimmune conditions. The NLRP1 gene encodes leucine- rich repeat protein 1, a member of a group of cytoplasmic pattern recognition receptors, which recognize microbial lipopolysaccharide, thereby stimulating innate immunity. We hypothesized that polymorphisms in this gene may affect susceptibility to Autoimmune Addison`s disease and Type 1 Diabetes. The aim of this study was to analyse the associations of six NLRP1 SNPs (Single- Nucleotide Polymorphisms) with AAD and T1D within a Polish cohort. The study comprised 101 AAD, 221 T1D patients and 254 healthy control individuals. Genotyping was performed by PCR-RFLP and PCR-SSCP methods. The minor allele of the coding SNP rs12150220 appeared significantly more frequently in AAD compared to healthy individuals (OR=1.5, 95% CI: 1.08-2.08, p=0.015). The distribution of genotypes also demonstrated significant differences. The frequency of high-risk genotype AA of rs12150220 SNP was significantly increased among AAD subjects vs. controls (P=0.006) yielding an OR of 2.96 (95%CI 1.34-6.55). Likewise, the heterozygous genotype TA was observed more frequently in the patient group [OR 3.09 (95%CI 1.53-6.24), P=0.001]. The frequencies of alleles and genotypes of the six SNPs in the NLRP1 region did not

present significant differences between patients with T1D and controls. In conclusion, this study confirms an association between the coding polymorphism in NLRP1 and AAD.

P09.197
Association genes for angiogenic factors and hereditary thrombophilia with preeclampsia in Russian and Yakut populations

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Several lines of evidence support the hypothesis that angiogenic factor and markers of hereditary thrombophilia play an important role in the pathogenesis of preeclampsia (PE). In the present study we examined 620 women (330 Yakut women [215 cases] and 290 Russian women [140 cases]). Total DNA was extracted, and 8 tagging single nucleotide polymorphisms in 3 genes (*VEGFA, ACVR2a* and *LEP*) and allelic variations C677T and A1298C in *MTHFR* gene, -675 4G/5G in *PAI-1* gene and G1691A in *FV* gene were genotyped. We have detected significant associations for PE with rs3025010 in *VEGFA* (OR=1,98; 95% CI: 1,20-2,36 for genotype TT), rs17742342 in *ACVR2a* (OR=1,68; 95% CI: 1,02-2,76 for genotype AA), rs2167270 in *LEP* (OR=2,38; 95% CI: 1,14-4,97 for genotype AA), genotypes 677TT in *MTHFR* (OR 8,17, CI 2,37-28,17) and -675 4G/4G in *PAI-1* (OR 2,36, CI 1,45-3,84) in Russian population. In Yakut population, we have found that only *PAI-1* 4G/4G genotype carriers have an increased risk of PE (OR 2,02, CI 1,29-3,16). Allelic variations in the *ACVR2a, LEP, MTHFR* and *VEGFA* genes are associated with PE only in Russian women, while polymorphism 4G/4G in *PAI-1* gene shows the association in both ethnic groups. We suppose that these polymorphisms could play a role in the development of PE. Nevertheless, the clinical significance of these findings remains to be determined.

P09.198
Genome-wide linkage and association analyses in nondemented hypertensive individuals suggest association of *PSEN2* with plasma amyloid beta

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Plasma amyloid beta (Aβ) is an easily obtained marker for Aβ load in brain. Aβ is highly correlated with hippocampal atrophy, a biomarker for early stages of late-onset Alzheimer's disease (AD). The aim of this study was to find genetic variants associated with plasma Aβ levels. The study included 126 nondemented hypertensive subjects 55 to 75 years old from the Erasmus Rucphen Family (ERF) study. Multipoint variance-component linkage analysis was performed for non-fasting plasma Aβ levels (Aβ40, Aβ42, and truncated forms Aβn40 and Aβn42) in MERLIN. Regions with at least suggestive evidence of linkage were followed up with association analysis using FASTA approximation to linear mixed model in GenABEL. All analyses were adjusted for age and sex. Two regions showed LOD scores >1.9, both for Aβ40: 1q32.3-1q42.13 (LOD = 2.07 at 1q41) and 11q14-11q21 (LOD=2.97 at 11q14.3). At 1q42.13, strongest association was observed at a variant flanking the *PSEN2* gene (p-value $2.5x10^{-4}$). Suggestive association was also observed at 11q14.3 (p-value=$4.8x10^{-3}$) at a variant near the Melatonin Receptor 1B (*MTNR1B)* gene. Our data suggests an association between *PSEN2*, a known gene for familial AD, and plasma Aβ40. The 11q14.3 region for Aβ40 contains the *MTNR1B* gene associated with obesity - a trait linked with plasma Aβ42 levels. Melatonin has an anti-apoptotic effect in neurodegenerative disease in cell and animal models. This region is therefore particularly interesting for further research. Replication efforts in a population-based sample are ongoing.

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P09.199
Evaluation of APOE, MTHFR and LOXL1 polymorphisms in pseudoexfoliation syndrome and pseudoexfoliation glaucoma in Epirus (Greece).

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Aim: The purpose of this study is to evaluate *APOE*, *MTHFR* and *LOXL1* polymorphisms as genetic risk factors for pseudoexfoliation syndrome (PXFS) and pseudoexfoliation glaucoma (PXFG) in the Greek population of Epirus.
Material & Methods: Genomic DNA was extracted from 82 PXFG, 69 PXFS and 96 control subjects from Epirus, Greece. *APOE* and *MTHFR* 677C>T genotyping was performed by polymerase chain reaction (PCR), followed by HhaI /HinfI digestion, respectively, and gel electrophoresis. A real-time PCR method with hybridization probes was developed for two *LOXL1* polymorphisms in a single reaction in the LightCycler (Roche-TIB).
Results: No significant differences were observed for the *APOE* and *MTHFR* polymorphisms between PXFS, PXFG and control subjects. The *APOE* e3 allele was found to be the commonest in all groups (88.4%-90.2%-89.1% in PXFS-PXFG-controls). The prevalence of the *MTHFR* 677TT genotype was 13.1%-14.6%-19% in PXFS-PXFG-controls. The *LOXL1* developed method was in 100% concordance with DNA sequencing. The genotyping of the two *LOXL1* polymorphisms is in progress.
Conclusion: Our results suggest that the *APOE* and *MTHFR* polymorphisms are not significant risk factors for the development of PXFS and PXFG. The results of the genotyping of the two *LOXL1* polymorphisms are expected.

P09.200
Serotonin gene receptor (5HT3a) expression in psoriatic patients

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Serotonin (5HT) plays roles as a neurotransmitter and a neuromodulator with functions in brain and peripheral tissues. Serotonin receptor subtypes are related to depression and also have been shown to be present in certain cells of the immune system. Psoriasis is a hyperprolifrative inflammatory disease. The aim of this study is evaluation of 5HT3a gene expression in peripheral blood mononuclear cells (PBMC) of psoriatic patients in comparison with normal individuals By Real-time PCR.
The PBMC was separated from whole blood by Ficoll-hypaque. The total cellular RNA was extracted and the cDNA was synthesized. This process was followed by real-time PCR using primer pairs specific for 5HT(3a) serotonin receptor mRNA and beta-actin as internal control. Results reveal that relative expression of 5HT(3a) has significant decrease in psoriasis. Considering these results the present study has shown that there is a change in serotonin receptor gene expression in PBMC of psoriatic patients in comparison with healthy individuals. In conclusion, 5HT3a significant decrease may be an important role of this receptor in pathogenesis of psoriasis.

P09.201
Targeted resequencing of susceptibility genomic regions for psoriasis

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Many recent publications have shown that DNA enrichment in combination with high-throughput sequencing can be successfully applied for the identification of causative genetic variants underlying both rare and common disorders. Psoriasis is a hyperproliferative inflammatory disease that presents several clinical forms and affects the skin, scalp, nails and joints. It is a life-long disease with a prevalence of 2-3% in the general population. The importance of the genetic factors for the susceptibility to psoriasis is supported by the high heritability of the disease, and also by several genome-wide linkage analyses and association studies that have identified multiple loci associated to the susceptibility for psoriasis. However, not all the genetic variants of these regions have been analysed with the same detail.

Therefore, we have re-sequenced six psoriasis candidate genomic regions on ten unrelated patients. The regions of interest were captured with the SureSelect (Agilent) target enrichment technology and sequenced using the Illumina GAII platform. The resulting reads were aligned to the reference sequence. After variant calling, we applied a combination of filters based on frequency and function to discard any variation that is not linked to the disease, and to define a set of candidate protein-altering variants to be associated to psoriasis. This analysis has allowed us to detect more than 300 hundred SNPs and InDels (both novel and known) in 78 different genes. The potential involvement of these variants in psoriasis is currently being evaluated through association studies in additional patients.

P09.202***
Imaging Genetics: tool for gene finding and characterization in psychiatric disorders

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Alteration of regional brain structure is an inherent feature of neuropsychiatric disorders, but it remains unclear if this is causally related to pathogenesis. If this were the case, alterations of brain structure should also be present in healthy individuals carrying genetic risk factors for such disorders. This, in turn, would imply that brain structure in the healthy population can be used as an intermediate phenotype for gene finding in neuropsychiatric disorders.

We enrolled >1700 young healthy adults in the Brain Imaging Genetics (BIG) study. Structural MRI brain images are available from scans at 1.5 or 3 Tesla. FSL-FIRST is used to assess volumes of specific brain structures. Genotyping of several candidate genes and genome-wide genotyping (Affymetrix 6.0, n=1000) was performed.

Hypothesis-driven testing of candidates for neuropsychiatric disorders lead (among others) to the following findings: By gene-wide (set-based) testing of the Alzheimer's disease (AD) gene SORL1 we found a significant association with hippocampus volume, one of the first structure affected by AD, in a discovery sample of 446 BIG-participants (p=0.0432) and replication in 490 additional subjects (p=0.0093).

Preliminary GWAS of amygdala volume in 600 samples resulted in association findings for several suspected psychiatric risk genes, such as CDH13, featuring among the GWAS top-findings for ADHD, addiction and schizophrenia.In conclusion, risk factors for neuropsychiatric disorders indeed affect brain structure in healthy individuals suggesting that altered brain structure is cause rather than consequence of disease. In addition, healthy brain structure seems a powerful intermediate phenotype for gene discovery in neuropsychiatric disorders.

P09.203
Support for the involvement of the calreticulin gene in the evolution of cognition in humans

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Development-dependent, tissue-specific expression of calreticulin (CALR) gene in the gray matter coincides with the expression of psychoses phenotypes. We have recently reported instances of mutations within the core promoter sequence of the gene in schizoaffective disorder. In view of the mounting evidence on the genetic overlap in the psychiatric spectrum, we investigated this gene in a spectrum of patients afflicted with schizophrenia, schizoaffective disorder and major affective disorder. We found that a unique mutation at nucleotide -220 from the transcription start site, located at a conserved genomic block in the promoter region of the gene, co-occurs with the spectrum of psychoses ($p<0.005$). This mutation reverts the human promoter sequence to the ancestral types observed in chimpanzee, mouse, and several other species, implying that the genomic block harboring nucleotide -220 may be involved in the evolution of human-specific higher-order functions of the brain (e.g. language, conceptual thinking, and judgment), that are ubiquitously impaired in psychoses. We propose that CALR is not only a promising candidate in the spectrum of psychoses, but also, a gene that may be important in the human-unique brain processes.

P09.204
Molecular characterization of 30 Pulmonary Arterial Hypertension idiopathic Spanish patients

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Pulmonary arterial hypertension (PAH) is characterized by an increase in PA pressure, increased pulmonary vascular resistance, vascular remodeling, and right ventricle failure. Approximately 70% of patients with the familial form of PAH have a mutation in the gene encoding bone morphogenetic protein receptor type II (BMPR-II). However, there is variable phenotypic expression of PAH among carriers of mutated BMPR-II gene. Likely related to a second hit caused by environmental and/or genetic modifiers. Studies have implicated serotonin, the serotonin transporter, endothelin, nitric oxide sintase, and others.

Vasodilators such as nitric oxide (NO) and prostacyclin, along with prolonged overexpression of vasoconstrictors such as endothelin (ET-1) or serotonin (5-HT), not only affect vascular tone but also promote vascular remodelling. All of them have been implicated in the pathogenesis of PAH.

NO is an endothelial-derived relaxing factor that is synthesized from L-arginine by nitric oxide synthase. NOS2 is the major source of NO production and polymorphisms in the *NOS2* gene promoter are thought to regulate its transcription activity.

The mitogenic and co-mitogenic effects of 5-HT require internalization through the serotonin transporter 5-HTT (*SERT*).

The aim of this work was to investigate the association between polymorphisms in the genes *ET-1, NOS2, SERT* and *BMPR-II* (by PCR-Sequencing) and the susceptibility to PAH.

We found 15% of sequence variations in the BMPR-II gene in the HAP patients. The data reveals that the shorter forms of the CCTTT repeat in the *NOS* gene were associated with susceptibility to PAH. No relation has been found with *ET-1* and *SERT*.

P09.205
Analysis of renin-angiotensin-bradykinin system genes polymorphism in preeclampsia, hypertension and metabolic syndrome in children of North-west Russia

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Renin-angiotensin-bradykinin system (RAS) is a pivotal regulator of arterial blood pressure and water-salt balance in human. It was shown gene polymorphisms of this system is associated with hypertension. The main aim of this study was the analysis RAS genes polymorphism in patients with preeclampsia, hypertension, children with metabolic syndrome. Population group (N=200), children (N=144), children with metabolic syndrome (N=160), children with hypertension (N=179), normal pregnant women (N=100), women with preeclampsia (N=100) were studied by PCR-biochip method including polymorphism of AGT (Met235Thr), AGTR1 (1166A>C), AGTR2 (3123C>A), BDKRB2 (-58T>C), REN (-83G>A) genes. We compared the results in patients groups with relevant data from controls. Only genotypes frequency of AGTR1 polymorphism (1166A>C) was different in children with hypertension from corresponding control group (x2=8.24; p<0.02). Further studies are needed to assess this finding.

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P09.206
Genome-wide association study reveals two novel susceptibility loci for restless legs syndrome

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Restless legs syndrome (RLS) is a sleep-related sensorimotor disorder with an age-dependent prevalence of up to 10% in the general population (> 65 years). It is characterized by the presence of uncomfortable sensations and an urge to move in the lower limbs, occurring in resting situations during the evening or at night. Relief of the symptoms is provided by moving the legs.

Genome-wide association studies (GWAS) have identified a total of four susceptibility loci: *MEIS1*, *BTBD9*, *MAP2K5/SKOR1* and *PTPRD*. However, the number of samples in the GWA stage was small, enabling the discovery of large effect (OR ≥ 1.4) common variants only. We conducted an enlarged GWA for RLS in 922 cases and 1,526 controls, followed by a replication of 76 candidate SNPs in 3,935 cases and 5,754 controls, all of European ancestry.

Herein, we identified two novel susceptibility loci: an intergenic region on chromosome 2p14 (rs6747972, P_{nom}=8.09 x 10^{-11}, OR= 1.2) and a locus on 16q12.1 (rs3104767, P_{nom}=1.96x10^{-19}, OR= 1.3) in a linkage disequilibrium block of 140 kb containing the 5'-end of *TOX3* and the adjacent non-coding RNA *BC034767*. *TOX3* is involved in calcium-dependent neuronal transcription, whereas for *BC034767*, a regulatory function can only be hypothesized at present.

In combination with the four known loci, all susceptibility variants explain about 3.4% of the heritability for RLS, clearly pointing to many more variants remaining to be discovered, until one can sketch the underlying molecular pathways.

P09.207
A copy number variant that provides a 2.3-fold protection against Rheumatoid Arthritis

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The genetic component of Rheumatoid Arthritis (RA) risk is estimated at around 60%, a large proportion of which is currently unaccounted for. In part this is due to that fact that, aside from the MHC, RA susceptibility loci so far described have small effect sizes (OR ≤ 1.8). Here we report an allele of a copy number variant (CNV) which is protective against RA, with an odds ratio of 2.3.

This CNV involves part of a large ancient tandem duplication, located within an RA susceptibility region. We assessed copy number in a Swedish RA cohort (cases = 2403, control = 1269), and revealed that a 129 kb deletion within this region occurs at a significantly higher frequency in control samples compared to cases (p = 0.001, OR = 2.3 (95% CI 1.4 - 3.9)), indicating a role protective against RA for this variant. This was replicated in a UK cohort (p = 0.036, OR = 1.90 (95% CI 0.93-3.82)).

Genes within this tandem duplication are involved in important biological processes including the immune response and embryogenesis. Altered expression of these genes has also been implicated in the progression of diseases such as cancer and Alzheimer's Disease. Given the importance of genes within this region, it seems likely that this CNV contributes towards susceptibility for other complex disorders. Indeed, our preliminary investigations have already suggested an association with psoriasis in a Swedish cohort (p=0.013, OR=2.16 (95% CI 1.2-4.1)).

P09.208
Analysis of eNOS gene polymorphisms in Serbian patients with rheumatoid arthritis

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Nitric oxide (NO), synthesized from L-arginine by NO synthases (NOS). To date, several functionally relevant genetic polymorphisms in the eNOS gene have been associated with various vascular and autoimmune diseases, such as rheumatoid arthritis (RA). The objective of this study was to investigate the influence of the eNOS gene polymorphisms (T-786C in the promoter, intron 4 a/b VNTR and G894T in exon 7) on the susceptibility to RA in the Serbian population. A group of 194 RA patients and 104 healthy subjects were included in this study. The genotype was determined by PCR-RFLPs method.

Our results showed that T-786C genotype distributions in RA patients (TT 46,1%, CT 45,0% and CC 8,9%) were significantly different from healthy controls (TT 49,4%, CT 50,0% and CC 0,96%) (χ2=7,335, p<0.05). We have found a higher frequency of -786C allele in RA patients compared to the controls but without statistically significant association (31, 4% versus 25, 9%) (χ2=2,142, p<0.05, OR=1, 28, and 95%: 0,858-1, 920). Group of patients with -786CC genotype had statistically significant lower number of swollen and tender joints compared with patients with -786TT genotype (t=1,672 p=0,039).

No significant difference in alleles or genotypes frequencies for the 4 a/b VNTR and the G894T polymorphisms was observed between RA patients and controls. Also, no significant differences in three eNOS allele frequencies between groups of patients according to immunological (RF, ANA) and other clinical data were observed. We conclude that there is potential association of C-786T polymorphism with the susceptibility to RA.

P09.209
Association of rs10818488 SNP at TRAF1/C5 region with rheumatoid arthritis in Iranian population

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Rheumatoid arthritis (RA) is a common autoimmune disease with a complex etiology affecting 1% of the world population. Association studies in various populations have reported a number of genetic variations affecting the individual susceptibility to RA. The strongest association has been reported from genes within the HLA region, particularly the HLA-DRB1 gene.

The association of rs10818488 SNP located near TRAF1 gene, has been recently picked up by genome wide association studies. Following independent studies in different populations revealed inconsistent results. To investigate the possible association of this SNP with RA in Iranian population, a combined family-based (parents-affected child

trios) and population-based case-control study was performed.
In family-based study, 115 trios were recruited. After obtaining their consent, blood samples were taken, and DNA was extracted. Genotyping was done by PCR-RFLP and a set of genotypes were confirmed by sequencing. Analysis of data showed a significant over-transmission of A allele in trios (Transmission Disequilibrium Test, TDT value ≈ 4.3, p-value ≈ 0.03). This resulted in a Haplotype Relative Risk of 1.5.
In case-control design, a total of 362 cases and 422 healthy controls, including samples from family-based study, were genotyped. Genotype and allele frequencies were compared between the two groups. Analysis showed a higher frequency of A allele in cases, although the difference was not significant statistically (Chi-square ≈ 2.8, p-value ≈ 0.09). Comparison of genotype frequencies, revealed higher frequencies of AA and AG genotypes in case group but statistically the difference was not significant (Chi-square ≈ 2.72, p-value ≈ 0.25).

P09.210
The rs2476601 SNP of PTPN22 gene plays no role in susceptibility to rheumatoid arthritis in Iranian population

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Rheumatoid arthritis (RA) is a common autoimmune disease with a complex etiology affecting 1% of the world population. Association studies in various populations have reported a number of genetic variations affecting the individual susceptibility to RA. The strongest association has been reported from genes within the HLA region, particularly the HLA-DRB1 gene.
The rs2476601 SNP of PTPN22 (protein tyrosine phosphatase nonreceptor 22) gene can be named as the second polymorphism that has been repeatedly reported to be associated with RA. Allelic frequency of 14 - 15% has been reported for A allele (the risk causing allele) in this SNP in Caucasian population. To investigate the possible association of this SNP with RA in Iranian population, we designed a family-based (parents-child trios) and a population-based case-control association studies.
In total 872 blood samples were collected from 405 confirmed RA patients and 467 healthy controls. 115 trios were included in the total samples. DNA was extracted and genotyping was performed by PCR-RFLP method using RsaI restriction enzyme. All samples were genotyped as homozygous GG. Genotyping was repeated for 30% of samples by using XcmI enzyme and for 10% of samples by sequencing. Again all samples showed homozygous GG genotype.
This study strongly suggest that there is no A allele in the gene pool of Iranian population. Therefore it does not play a role in susceptibility to RA and other autoimmune disorders in Iranian population.

P09.211
Strengthening the Reporting of Genetic Risk Prediction Studies: The GRIPS Statement

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The rapid and continuing progress in gene discovery for complex diseases is fuelling interest in the potential implications of this knowledge for clinical and public health practice. An essential prerequisite for many genome-based health care applications is the predictive ability of the genetic risk model. The number of studies assessing the predictive performance of genetic risk models is steadily increasing, with widely variable completeness of reporting and apparent quality. Transparent reporting of the strengths and weaknesses of empirical studies is important to facilitate the accumulation of evidence on genetic risk prediction. A multidisciplinary panel developed a checklist of 25 items recommended for strengthening the reporting of Genetic RIsk Prediction Studies (GRIPS), building on the STREGA, REMARK and STARD reporting guidelines. The recommendations do not prescribe or dictate how genetic risk prediction studies should be conducted, but offer consensus guidelines to enhance the transparency of their reporting, and thereby to improve the process of synthesizing information from multiple studies with different strategies regarding design, conduct or analysis.

P09.212
ELP4 polymorphisms and rolandic epilepsy in a Greek cohort

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Rolandic epilepsy (RE) is the most common idiopathic focal epilepsy in childhood characterized by an EEG abnormality of centrotemporal sharp waves. Results from a recent genome-wide linkage analysis have implicated the Elongator protein complex 4 (ELP4) gene, in the pathogenesis of this neurodevelopmental disorder. ELP4 is a component of the elongator complex which regulates the transcription of several genes associated with nerve cell motility and migration during development, however its role is not completely defined up to now.
In an attempt to investigate the previously reported association of ELP4 polymorphisms, rs964112 and rs11031434, with RE, we collected 31 Greek patients (age range: 4-16 years old diagnosed in the AHEPA University Hospital of Thessaloniki, Greece and 47 healthy controls.
The polymorphisms were detected with a PCR-RFLP method, after designation of specific primers. PCR amplification and enzyme digest of the relevant fragment.
Genotype and allelic frequencies were compared with the Chi-square test of independence. Genotype and allele distributions for the two studied polymorphisms were not significantly different between RE and control individuals (for genotypes: P=0.225 and P=0.828 respectively, for alleles: P=0.852 and P=0.993, respectively). Haplotype frequency comparisons did not show any significant difference between RE patients and controls (P=0.649), as well.
Our results do not substantiate ELP4 as a risk locus for Rolandic epilepsy, although an extension of this study to a larger sample is expected to delineate the effect of ELP4 genetic variations on the susceptibility to seizures and neurodevelopmental disorders, such as RE.

P09.213
rs1333040 genotypes on 9p21.3 influence CDKN2A/2B expression in human coronary plaques

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Coronary artery disease (CAD) is significantly influenced by genetic background. Genome-wide association studies have shown that common genetic variants located in human chromosomal region 9p21.3 are associated with ischemic heart disease. They are not associated with any established cardiovascular risk factors, and no known protein-coding genes reside within the region. Gene expression studies have shown that gene regulatory elements might be located within the risk interval.
The knock-out mice lacking the CAD locus interval in chromosome 4 shows that this region is critically required for the normal cardiac expression of two neighbouring cyclin-dependent kinase inhibiting genes, Cdkn2a/b.
It is known that CDKN2A/B control cell proliferation by preventing entry to the S-phase of the cell cycle. As the development of human atherosclerotic lesions is characterised by an increase in the rate of replication and death of all mitotic cells in response to injury, it is conceivable that the decreased expression of CDKN2A/B may be associated with altered cell replication, and the start and progression of atherosclerosis.
We investigated the influence of the 9p21.3 variant rs1333040 on CDKN2A/B expression in 30 human coronary atherosclerotic plaques obtained by means of coronary atherectomy from patients with ischemic heart disease. We validated our findings on 20 coronary samples obtained at the heart transplant.

Quantitative PCR showed a significant (p=0.00013) trend of expression levels of both genes with the number of the risk allele (C), the expression in the CT plaques being in between that of CC and TT plaques, for both genes.

P09.214
Proteome screening in sera of patients with schizophrenia at different stages and courses of drug therapy

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Schizophrenia is a severe chronic mental disorder that affects most of the higher brain functions. Its prevalence is estimated on about 1% worldwide. The classical symptoms also occur in several other psychiatric disorders, which makes difficult the exact diagnostics. The etiology of schizophrenia remains to be elucidated. It is thought to be a result of a complex interaction between genetic and environmental factors. Schizophrenia is a serious social and economic burden to healthcare systems. Currently, there is no etiological treatment. Until now there isn't any proven molecular marker as an objective diagnostic parameter for schizophrenia.

Materials and methods: We have analyzed serum samples from schizophrenic patients at different phases of the disease - before and after treatment, comparing them to a cohort of normal sera. We applied several methods for protein alterations' screening: DSC (Differential scanning calorimetry), protein profiling methods: 2D-SDS PAGE, HPLC techniques. The differences in the profiles were analyzed by mass spectrometric techniques.

Results: Initially we analyzed sera samples with DSC method to detect distinguishing serum profiles. We observed significant differences in the fractions of the more abundant serum proteins- albumin and immunoglobulin. We distinguished different protein profiles: before and after treatment of the patients; according to the medication. We also found considerable concordance between therapy response and protein profiles - normalization in case of remission and marked alterations in non-responders. Because of these differences we proceeded with analyzing serum proteins by more sensitive and precise methods to find a potential marker for drug therapy and disease progression.

P09.215**
Increased exonic de novo mutation rate in probands affected with schizophrenia

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Schizophrenia (SCZ) is a severe psychiatric disorder that profoundly affects cognitive, behavioral and emotional processes. The wide spectrum of symptoms and clinical variability in SCZ suggests a complex genetic etiology, which is consistent with the numerous loci thus far identified by family linkage, copy number variation (CNV) and association studies. While SCZ heritability may be as high as ~80% the genes responsible for much of this heritability remain to be identified. Using high-throughput sequencing technologies, we sequenced the exome of 14 patients with schizophrenia and their parents. Each proband had a standardized SCZ diagnostic and every parent was carefully excluded of any psychiatric disorder. A list of high quality variant was validated using traditional Sanger sequencing. We identified 14 DNMs in 8 probands, which is significantly more than expected considering the previously reported DNM rate. Beside, 4 of the 14 identified DNMs are nonsense mutation, suggesting a causal role for those variants. Our study supports the notion that DNMs play a major role in sporadic SCZ while providing a list of genes possibly involved in disease pathogenesis. This report suggests that de novo mutations (DNMs) may account for a substantial fraction of the missing SCZ heritability.

P09.216
Expression QTL analysis of top loci from Psychiatric GWAS Consortium meta analysis reveals additional schizophrenia candidate genes

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Schizophrenia is a severe psychiatric disorder characterized by delusions and hallucinations, in addition to negative and cognitive symptoms. The disorder affects up to 1% of the population and the genetic contribution is estimated to be around 80%. Thus far, genome-wide association studies of schizophrenia have had limited success, with the best finding at the HLA locus at chromosome 6p. While only a very small number of loci yield genome-wide significance for association, the vast majority of the genetic contribution to disease susceptibility remains uncovered. It is likely to be overrepresented in non-significant top SNPs of large-scale GWAS results. In order to uncover potential interesting candidates, we selected the top 6,000 SNPs with significance threshold at p<0.001 from the international PGC meta-analysis consisting of some 9,000 cases and 12,000 controls and examined their possible involvement in schizophrenia using gene expression data from whole blood. Expression QTLs were calculated for this selection of SNPs in a set of healthy controls (n=437). The transcripts significantly regulated by the top SNPs from were subsequently tested for differential expression in an independent set of schizophrenia cases and controls (n=200). The eQTL analysis yielded significant cis-acting effects, whereas no trans effects were observed. For seven of these transcripts we observed significant differential expression between cases and controls. Results include target genes of interest both in- and outside the HLA region and contain genes that are known to be expressed in brain. These genes are strong candidates for schizophrenia for which further genetic analysis is warranted.

P09.217
Altered expression of Peroxysome proliferator-activated receptor Gamma 2 and serum leptin levels in schizophrenia

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It has long been known that schizophrenia (SCH) is associated with morbidity and mortality rates that far exceed those of the general population. Leptin receptor (LEPR), leptin (LEP) and peroxysome proliferator-activated receptor γ2 (PPARγ2) are the potential genetic determinants which might be liable of metabolic dysregulation in SCH patients. The aim of this study was to evaluate the effect of LEPR gene p.Q223R polymorphism and the impact of mRNA levels of LEPR, LEP, and PPARγ2 along with the serum leptin levels on metabolic adversities in SCH patients (n=80) and controls (n=115).

Methods: Metabolic profiles, LEPR gene polymorphism and the gene expressions of LEPR, LEP and PPARγ2 were studied in SCH patients and controls.

Results: A significant difference was determined between SCH and control groups concerning genotype frequency of LEPR p.Q223R (OR=10.9 95%CI=4.7-25.2) (p=0.000). LEPR p.Q223R was strongly associated with waist circumference (p=0.003) in SCH patients. LEP and LEPR gene expressions and serum leptin levels were higher in SCH patients compared to controls. PPARγ2 gene expression and serum leptin levels were significantly increased in SCH patients carrying LEPR p.Q223R polymorphism (QR+RR) (p=0.003 and

p=0.001, respectively) (Table1).
Conclusion: Leptin receptor, leptin and PPARγ2 genes could be potential risk factors in developing metabolic adversities in SCH.
Table 1: Clinical ,biochemical and genetic characteristics of SCH patients with LEPR p.Q223R genotypes

Traits	Total, mean (SD)		
p.Q223R	QQ n=8	QR+RR n=72	*P* values
Age (years)	34.5±5.07	30.4±7.05	0.188
BMI (kg/m2)	24.4±2.7	25.3±3.5	0.399
Weight (kg)	70.7±11.1	75.3±12.1	0.773
Waist circumference (cm)	94±5.5	111.7±13.1	***0.003***
Fasting glucose (mU/L)	85.3±12.8	80.7±14.6	0.554
TC(mg/dl)	188±34.4	187.2±37.8	0.968
TG(mg/dl)	136.3±70	156.9±72.7	0.608
HDL-C(mg/dl)	47±6.1	42.3±6.3	0.193
SBP(mmHg)	130±8.2	120.1±15.28	0.091
DBP(mmHg)	80±14.1	76.1±12.7	0.581
Leptin (ng/ml)	0.3±0.01	1.77±1.5	***0.001***
PPARG expression (Arbitrary Unit)	1.27±0.48	2.6±1.7	***0.003***

P09.218
serotonin receptor genes as markers of intellectual abilities

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Important focus in human genetics is the study of genetic determination of individual-personality characteristics, which primarily include a person's ability, so at the forefront search of genetic markers that define these abilities.There is evidence that, for gifted children is characterized by a more rapid transmission of neural information that may be related to the activities of neurotransmitter systems, a special place among them being the serotoninergic system.Serotonin receptor genes HTR2A, HTR1A, HTR2C are one of the major genes determined work of the serotoninergic neurotransmitter systems, and their functional status can be reflected on some aspects of human intellectual activity.Material were DNA samples 184 people.We used cultural-free Kettell test.To analyze the association polymorphisms of serotonin receptor genes with the level of intellectual development was investigated sample was divided into groups according to the gradation level IQ: low- below 90, average- 90-139, and high - above 140.In the group with a high level of intellectual development showed an increase in the frequency of alleles of HTR2A * A (P = 0.002) and genotype of HTR2A * A / * A (P = 0.04) in the serotonin receptor gene HTR2A.In the group with low levels of intellectual development showed an increase in the frequency of alleles of HTR2A * G (P = 0.002) in the gene for serotonin receptor type 2A, as well as genotype HTR1A * G / * G (P = 0.002) in the gene for serotonin receptor HTR1A.This work was funded grant RHSF 10-06-84.609.

P09.219
Serum Hepcidin levels and association studies of TMPRSS6 and HFE variants provide further insights into regulation of iron homeostasis

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Genetic variants of hepcidin-regulatory genes, *TMPRSS6* and *HFE*, affect erythrocyte traits and iron parameters, namely serum iron and transferrin saturation in normal populations. To analyze whether this effect is mediated by the iron regulator hepcidin, we measured serum hepcidin levels in 1657 genotyped individuals from an Italian cohort, the Val Borbera (VB) population. Hepcidin levels showed age and sex dependent variations that correlated with ferritin but not with serum iron or transferrin saturation. We replicated the observation that common variants of the *HFE* (rs1800562, C282Y) and *TMPRSS6* (rs855791,V736A) genes are associated with serum iron and transferrin saturation, but no association was found with hepcidin. Both variants showed a modest effect on the hepcidin/ferritin ratio, and the association was enhanced once iron-deficient and inflammation-positive samples were excluded from the analysis. Interestingly, in such a subset of samples, *HFE* C282Y was associated to ferritin at genome wide significance and the association was further enhanced after adjusting for hepcidin levels. Our results suggest that the main effect of *HFE* C282Y variant is to increase cell iron uptake through increased transferrin saturation. The effect is in part counterbalanced by impaired hepcidin levels which favor cell iron release, especially from macrophages, in this way likely reducing, serum ferritin levels. Finally we show that the effect of *HFE* and *TMPRSS6* variants on erythroid traits is mainly mediated by variations of iron parameters, but not by variations of hepcidin.

P09.220
Differential analysis of molecular profiles between schizophrenia and bipolar affective disorder after genome-wide association study in Bulgarian population

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The major psychotic illnesses - schizophrenia and bipolar disorder, are among the most common debilitating mental conditions that constitute some of the leading causes of disability and pose significant economic burden to society. Although genetic factors make substantial contributions to their etiology, the identification of specific susceptibility genes for both disorders has not been straightforward. In recent years new molecular genetic findings have implicated risk factors for these disorders, thus allowing the possibility of a genetic relationship between them to be directly explored. Findings from genome-wide association studies suggest a partial overlap in the genes that contribute to the susceptibility to schizophrenia and bipolar disorder. To test this statement we have compared our results from genome-wide association studies on bipolar disorder and schizophrenia conducted in 188 BAD patients, 188 schizophrenia subjects and 376 healthy Bulgarians. The analysis of top 100 polymorphisms revealed no genetic overlap between two conditions in our population. The strongest finding in bipolar disorder was not among the top findings after GWAS on schizophrenia. An SNP which revealed the most significant association with schizophrenia did not show any association with bipolar disease. Interestingly, the genome-wide scan on schizophrenia has detected a variant in a gene, which has already been reported as the strongest finding in previous genome-wide association scan on bipolar disorder. Although no genetic overlap between schizophrenia and bipolar disease has been detected in Bulgarian population, this finding supports the hypothesis that common genetic factors may be involved in the etiology of both conditions.

P09.221
Exercise induced steroid dependent dystonia, ataxia, and alternating hemiplegia associated with epilepsy: a novel phenotype associated with an *SLC2A1* mutation

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A 20 year old woman with a combination of a moderate learning disability, seizures, ataxia, exercise-induced dystonia and episodes of alternating hemiplegia was described by Neville et al. in *J Neurol Neurosurg Psychiatry* 1998;65:241-244 (Neville, 1998). Prednisolone improved the ataxia and was associated with cessation of seizures and exercise-induced dystonia. At the time, no diagnosis could be made. Over the last years, the clinical spectrum of *SLC2A1 (GLUT1)* mutations has been expanding. Apart from the classical phenotype which consists of drug resistant seizures, mild to severe developmental delay, and complex movement disorders, such as ataxia and dystonia several non classical presentations have been described as well. Recently, a child with episodes of hemiplegia was found to have a mutation in the *SLC2A1* gene (Rotstein, Neurology 2009). The combination of mental retardation, atypical absences, movement disorders with exercise-induced dystonia and alternating hemiplegia in this patient directed us to the possibility of an *SLC2A1* mutation. Mutation analysis of all 10 exons of the *SLC2A1* gene revealed a novel heterozygous missense mutation in exon 2. The parents did not carry the mutation, indicating that Gly18Arg is a de novo mutation. False paternity was excluded by genetic, multimarker analysis. Screening of 150 subjects from the general Dutch population was performed by sequence analysis of exon 2 and was negative. The Gly18Arg mutation is located in the first transmembrane segment of GLUT1 and is likely to cause disruption of the glucose transport across the aqueous central channel. The Gly18Arg mutation is therefore likely to be pathogenic. This patient presented with symptoms that all have been described in patients with GLUT1 deficiency before, but to the best of our knowledge the combination of a moderate learning disability, epilepsy, ataxia, exercise induced dystonia**,** and alternating hemiplegia has not been associated with *SLC2A1* mutations before. This patient is the first to be described by this specific combination of symptoms and underlines the heterogenic phenotype associated with *SLC2A1* mutations.

P09.222
Bitter taste sensitivity and nicotine dependence in a Romanian population

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TAS2R38 belongs to the TAS2R bitter taste receptor gene family. Three polymorphisms have been described in relationship with differences in bitter taste perception of phenyl-thiocarbamide (PTC). It was hypothesized that taster status is related to smoking, as individuals who perceive PTC as bitter may find the taste of cigarettes aversive, and thus, present a reduced risk to become smokers and nicotine dependent, as compared to non-tasters.

In 226 middle aged (median age: 41 years) Caucasians (53.53 % males, 46.47 % females), we have followed the relationship between the bitter tasting phenotype and genotype (rs713598), respectively, and tobacco dependence as assessed by the NDSS (Nicotine Dependence Syndrome Score), HSI (Heaviness of Smoking Index) and Fagerström Test for Nicotine Dependence.

As compared to a group of non-smokers (n = 112), the percentage of smokers who could taste PTC was lower (57.96 %), and the inability to taste associated with an increased risk for smoking (chi^2 = 3.56, p < 0.05). In smokers, overall scores in the three systems of dependency presented significant association with the TAS2R38 polymorphism (p < 0.01); out of the various traits of tobacco dependence (drive, priority, tolerance, continuity, stereotypy), drive presented a significant correlation with the ability to taste PTC (t = 2.46, 95%CI: 0.14-1.17, p = 0.01).

In conclusion, PTC non-tasters may become more frequently smokers not avoiding the unpleasant taste of cigarettes; however, in those who become smokers, a preference for bitter taste may contribute to the development of dependency.

P09.223
ESR1 polymorphic variants in stroke patients from Ukraine

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Multiple data support the hypothesis of estrogen role in vascular function, lipid metabolism regulation and neuroprotection. Two single nucleotide polymorphisms in intron 1 of estrogene receptor alpha gene (*ESR1)* - c.454-397T>C (*PvuII*) and c.454-351 A>G (*XbaI*) - are thought to influence *ESR1* gene transcription and change cell sensitivity to estrogen-estrogen receptor complex action.

To establish possible involvement of *PvuII* and *XbaI* polymorphisms into stroke development we investigated this SNPs in: case group - patients with ischemic stroke (n=183, men-95, women-88), control group I - individuals from the general population (n=100, men-50, women-50), control group II - healthy individuals elder then 65 years(n=88, men-35, women-53), from different regions of Ukraine. No significant difference between the age of men and women within each group was observed. Blood samples for DNA analysis were obtained after informed consent. Genotyping was performed by PCR followed by RFLP analysis. Obtained data has shown a linkage disequilibrium between studied alleles, CG and TA alleles are in phase (r^2 = 0,557).

We have found no differences in genotype or allelic frequencies between case group and control group I. Interestingly, difference in c.454-351G/G genotype frequency between women (33,7%) and men (20,4%) from case group was observed.

Frequency of c.454-351G/G genotype was higher in women from case group (33,7%) comparing to women from control group II (18%).

Differences were considered significant at P<0.05 value of Fisher exact test.

Our findings suggest a possible role of c.454-351A allele in stroke development in women.

P09.224
Analysis of association of GR gene haplotype and suicidal behavior in population from Bashkortostan (Russia)

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Suicidal behaviour (SB) is a complex trait for which the underlying pathophysiology has yet to be fully explained. Hyperactivity of the hypothalamic-pituitary-adrenal axis, leading to increased cortisol levels, has been suggested to play a role in the development of SB. The effects of cortisol, the most important glucocorticoid (GC) in humans are mainly mediated by the GC receptor (GR). Several polymorphisms in the GR gene that alter the GC sensitivity are known. The aim of this study was to study the role of these GR polymorphisms in SB. Three SNPs (9beta, BclI and N363S) in the GR gene were genotyped and haplotypes were constructed. We investigated a total sample of 636 subjects. Cases were 288 persons - hospitalized in the Clinical Republic Hospital (Ufa, Russia) after suicide attempt. Suicide attempts were classified as violent - hanging (4.2%), jumping from a high place or under a vehicle (6.3%), cutting (8.5%), or nonviolent - drug overdose (81%). Forty-seven patients (31.3%) admitted to previous suicide attempts. The most common primary diagnoses in the sample were: personality disorders (29.3%), depressive disorders (17.3%), substance dependence (16%), schizophrenia spectrum disorders (14.7%). Other diagnoses were: bipolar affective disorder (2%); posttraumatic stress disorder (1.33%). The control group consisted of 348 volunteers - without a personal or familial (first degree) history

of neuropsychiatric disorders and SB. The AGA haplotype (P=0.006, OR=1.50) was associated with increased risk of SB. This seems to result from the presence of BclI, and not from the 9beta and N363S polymorphisms.

P09.225

Estrogen receptor α and androgen receptor gene polymorphisms in patients with systemic lupus erythematosus and healthy controls

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Background: Systemic lupus erymathosus (SLE) is an autoimmune disease that affects mainly females. Disturbances of estrogen metabolism and decreased androgen levels were found in SLE, however the influence of hormone receptor genetic polymorphisms on the disease progress are still not clarified.

Methods: A total of 109 women with SLE and 47 female controls were genotyped for ER*α* polymorphisms PvuII T/C and XbaI A/G by RFLP analysis. The absence of PvuII and XbaI restriction sites were indicated by “P” and “X” and presence by “p” and “x”, respectively. The CAG repeat length in AR was also investigated by fluorescence-based fragment analysis on an automatic DNA sequencer. Shorter allele (SA), longer allele (LA) and biallelic mean (BM) were determined. SLE disease activity index (SLEDAI) and SLICC/ACR damage index were also calculated and relationships with the genetic parameters were investigated.

Results: No differences were found between the ER*α* alleles distribution in patients and controls. The CAG repeat lengths in the SA, LA and BM were also similar. The (CAG)n number of LA but not SA was inversely related to SLICC index (r=-0.207, p=0.031). In patients with PPXX genotype SLEDAI index was significantly lower than in PpXx patients (p=0.016) but not different in comparison to patients with ppxx (p>0.05).

Conclusions: Our preliminary results showed that AR and ER*α* polymorphisms are not crucial for the development of SLE but probably modulate the activity of the disease and chronic damages in patients.

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P09.226

The autoimmune disease-associated IL2RA locus is involved in the clinical manifestations of systemic sclerosis

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Objectives: Regulatory T cells (Tregs) are crucial in the maintenance of the immune tolerance and seem to play an important role in systemic sclerosis (SSc),. The interleukin 2 receptor alpha (IL-2RA) is an important Treg marker, and polymorphisms of IL2RA gene are associated with a number of autoimmune diseases. Therefore, we aimed to investigate for the first time the association of the IL2RA locus in SSc.

Methods: A total of 2983 SSc patients and 2652 matched healthy controls, from an initial Spanish cohort and five additional replication European cohorts of Caucasian origin, were genotyped for the IL2RA gene variant rs11594656 using the TaqMan® allelic discrimination technology.

Results: A significant association of IL2RA rs11594656*T with the presence of anti-centromere antibodies (ACA) was first detected in the Spanish cohort (P=6.30x10-3, OR=0.77). The combined analysis of the replication sets showed a clear association with limited cutaneous SSc (lcSSc, P=5.49x10-3 OR=0.84), and the overall meta-analysis including several cohorts evidenced that this variant is consistently associated with both lcSSc (P=1.71x10-3 OR=0.85) and ACA production (P=9.31x10-3 OR=0.86).

Conclusions: Our data strongly suggest that IL2RA is involved in SSc, specifically with lcSSc and ACA subgroups.

P09.227**

Identification of novel genetic markers associated with clinical phenotypes and auto-antibody subsets of systemic sclerosis through a genome wide association strategy

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The aim of this study was to determine the genetic components contributing to different systemic sclerosis (SSc) clinical sub-phenotypes of limited (lcSSc) and diffuse (dcSSc) cutaneous involvement, and with the SSc-specific auto-antibodies, anti-centromere (ACA) and anti-topoisomerase I (ATA) through a genome-wide association study (GWAS). Four GWAS cohorts, comprising 2,296 SSc patients and 5,171 healthy controls, were meta-analyzed looking for associations in the selected subgroups. Eighteen polymorphisms were further tested in nine independent cohorts comprising an additional 3,175 SSc patients and 4,971 controls. Conditional analysis for associated SNPs in the HLA region was performed to explore their independent association in antibody subgroups. Overall analysis showed non-HLA polymorphisms rs11642873 in IRF8 gene (P = 2.32x10-12, OR = 0.75) and rs12540874 in GRB10 gene (P = 1.27x10-6, OR = 1.15) to be associated with lcSSc and rs11047102 in SOX5 gene (P = 1.39x10-7, OR = 1.36) with ACA positive patients. In the HLA region, we observed highly associated allelic combinations in the HLA-DQB1 locus with ACA (P = 1.79x10-61, OR = 2.48), in the HLA-DPA1/B1 loci with ATA (P = 4.57x10-76, OR = 8.84) and in NOTCH4 with ACA P = 8.84x10-21, OR = 0.55) and ATA (P = 1.14x10-8, OR = 0.54). We have identified three new non-HLA genes (IRF8, GRB10, and SOX5) associated with SSc clinical and auto-antibody subgroups. Within the HLA region, HLA-DQB1, HLA-DPA1/B1 and NOTCH4 associations with SSc are likely confined to specific auto-antibodies. These data emphasize the differential genetic components of subphenotypes of SSc.

P09.228

Molecular investigation of TFAP2A and BMP4 genes in patients with cleft lip and palate

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Studies with mutant animals models can point directly to candidate genes in humans and have been contributing to the knowledge about pathogenesis of cleft lip and palate (CL+/-P). Mice with mutations in TFAP2A and BMP4 genes present the phenotype of clefts. In addition, mutations in BMP4 have been previously described in patients with nonsyndromic CL+/-P. Based on these evidence the aim of our study was to investigate the involvement of BMP4 and TFAP2A genes in the etiology of CL+/-P using mutation screening by direct sequence and enzyme digestion with HphI (PCR-RFLP). The sample included a total of 45 patients, 20 cases of syndromic CL+/-P and 25 cases of nonsyndromic CL+/-P. We found no pathogenic sequence variant in the TFAP2A gene. In the BMP4 gene, we found the rs17563 polymorphism (538T->C variant), previously described as associated with CL+/-P in the Chinese population. In order to investigate whether this same association exists in our sample, we genotyped an additional control group of 169 normal subjects with no antecedent of oral clefts in neither three generations nor oriental ancestrally. We found no significant differences in genotypes or allelic frequencies

between patients and controls (p=0.3347,OR=0.718255,95% CI: 0.4 to 1.27). However, further studies with larger samples are necessary to elucidate this aspect. Financial Support: FAPESP, CNPq

P09.229
Association of the toll-like receptor 4 Asp299Gly polymorphism with atopic dermatitis in Italian children.

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Background. Atopic dermatitis (AD) is a chronic inflammatory skin disease with a multifattorial pathogenesis. A genetically determined defective function of pattern recognition receptors such as toll-like receptors (TLRs) has been proposed as a candidate mechanism in the pathogenesis of AD.
Aim: To study the impact of genetic predisposition of TLR2 and TLR4 genes encoding for pattern recognition-related molecules for the phenotype of AD.
Methods. We performed a case/control study in which we included 102 Italian AD children (47 males and 55 females, median age 2,4 years) and 50 gender matched non-atopic, healthy individuals (27 males and 23 females, median age 2.5 years). The severity of AD was measured using the SCORing Atopic Dermatitis (SCORAD) Index for the evaluation extensiveness of skin disease. We examined the allelic frequencies of R753Q TLR2 and Asp299Gly TLR4 single nucleotide polymorphisms (SNPs). Genotyping was performed using PCR and restriction fragment length polymorphism analysis.
Results. For the R753Q TLR2 SNP, similar allelic frequencies were found in both groups. Moreover, the allelic frequencies of R753Q TLR2 SNP was no different between the groups with mild, moderate and severe SCORAD. A significantly increased frequency of the Asp299Gly allele TLR4 was present (p<0.001). This SNP was significantly associated with moderate and severe AD.
Conclusion. Our results suggest that in our group of children with AD, the TLR4 gene could play a crucial role in the development of AD, probably with other genetic factors.

P09.230
HRMA in the TPMT gene study in Polish population

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Thiopurine methyltransferase (TPMT) is involved in the transformation of thiopurine drugs which are used primarily in the treatment of autoimmune disorders such as inflammatory bowel disease (IBD). Gene TPMT encoding thiopurine methyltransferase is highly polymorphic. In our study we investigated the three most common TPMT alleles responsible for a decreased enzyme activity: TPMT *2 (p.Ala80Pro), *3A (p.Ala154Thr, p.Tyr240Cys) and *3B (p.Ala154Thr). These variants determine 80 - 95% of the intermediate and low enzyme activity, and increase the risk of thiopurine-induced toxicity. The aim of our study was to optimize the HRMA (High Resolution Melting Analysis) to identify main TPMT alleles as a simple alternative method to automatic sequencing and RFLP (Restriction Fragment Length Polymorphism). Moreover, we determined the frequency of these changes in Polish population. We tested 283 DNA samples of Polish population including 91 IBD patients treated with thiopurine drugs. Fragments of exon 4, 6 and 9 of TPMT gene enclosing codon 80, 154 and 240 respectively, were amplified and their melting profiles were analyzed using LightCycler® 480 equipment. The results were confirmed standard sequencing. Identification of sequence variants using HRMA is highly sensitive and less time consuming compared to standard sequencing or RFLP. It can be easily integrated into diagnostic testing. Furthermore, we found TPMT*3A allele frequency of 5,3 % in Polish population. The frequency of TPMT*3B allele in heterozygote is of 1,1%. TPMT*2 allele was not found.

P09.231***
A genome-wide meta-analysis of six type 1 diabetes cohorts identifies multiple associated loci

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Diabetes impacts approximately 200 million people worldwide, of which type 1 diabetes (T1D) represents approximately 10%. The application of genome wide association studies (GWAS) has robustly revealed dozens of genetic contributors to the pathogenesis of T1D, with the most recent meta-analysis identifying in excess of 40 loci. To identify additional genetic loci for T1D susceptibility, we examined associations in the largest meta-analysis to date between the disease and ~2.54 million SNPs in a combined cohort of 9,934 cases and 16,956 controls. Targeted follow-up of 53 SNPs in 1,120 affect trios uncovered three new loci associated with T1D that reached genome wide significance. The most significantly associated SNP (rs539514, P = 5.66x10-11) resides in an intronic region of the LMO7 (LIM domain only 7) gene on 13q22. The second most significantly associated SNP (rs478222, P = 3.50x10-9) resides in an intronic region of the EFR3B (protein EFR3 homolog B) gene on 2p23; however the region of linkage disequilibrium is approximately 800kb and harbors additional multiple genes, including NCOA1, C2orf79, CENPO, ADCY3, DNAJC27, POMC, and DNMT3A. The third most significantly associated SNP (rs924043, P = 8.06x10-9) lies in an intergenic region on 6q27, where the region of association is approximately 900kb and harbors multiple genes including WDR27, C6orf120, PHF10, TCTE3, C6orf208, LOC154449, DLL1, FAM120B, PSMB1, TBP and PCD2. These latest associated regions add to the growing repertoire of gene networks predisposing to T1D.

P09.232
The influence of polymorphic positions of the HLA-DRβ1 and HLA-DQβ1 allele heterogeneity on disease risk of type 1 diabetes mellitus in Iranian population

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Type 1 Diabetes mellitus (T1D) is a chronic autoimmune and multifactorial disease. The HLA-DRB1and DQB1 loci have the strongest association with T1D. Various HLA-DRB1and DQB1 alleles encode different amino acids in critical positions of HLA-DRβ1and DQβ1 molecules. In this study, we investigated highly polymorphic amino acid residues of HLA-DRβ1and DQβ1 molecules to determine their susceptibility or protective effect in100 Iranian T1D patients and 105 healthy controls. Analysis of the amino acid sequence of HLA-DRβ1 and DQβ1 chains revealed the $DRB\beta1^{Lys71+}$ (p: 10^{-14}; OR: 6.084) and the $DQ\beta1^{Asp57-}$ (p: 10^{-8}; OR: 3.31) were significantly more frequent in patients and had a positive association with T1D. The homozygous $DR\beta1^{Lys71+}$ (p: 2× 10^{-7}; OR: 30.938) and $DQ\beta1^{Asp57-}$ (p: 10^{-5}; OR: 4.111) had the strongest association with T1D susceptibility. According to our results indicating the crucial rule of the $DRB\beta1^{Lys71+}$, we designed allele-specific primers to develop an easy, fast and cost-benefit method to detect the $DR\beta1^{Lys71+}$. We also calculated PcPPV and PcNPV which showed that the person carrying the $DRB1^{Lys71+/+}$ genotype has 1% absolute risk to develop T1D.

P09.233
Two novel genes were identified as the susceptibility genes of the type 2 diabetes in Han Chinese

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Type 2 diabetes (T2D) is the forth leading cause of death in Taiwan. To identify susceptibility genes that increase the risk of T2D in Han

Chinese that accounts for 98% of the Taiwan population, we conducted a genome-wide association study in which 643 cases and 812 random controls were genotyped using Affymetrix SNP6.0 genechips. In the first stage for genome wide scan, we selected 75 SNPs in 43 regions that had-logP>4 with trend test for subsequent cross-plateform validation using Sequenom. After cross-plateform validation, a total of 72 SNPs in 40 loci yielded consistent results using both Affymetrix and Sequenom, including KCNQ1 and CDKAL1 loci previously reported to be associated with T2D. We took these SNPs forward to replicate in 785 additional samples (stage 2: 597 T2D cases and 188 controls). Of the 72 SNPs in 40 loci selected in stage 1, only two novel loci still showed a strong association in the joint analysis of stage 1 and stage 2, except previously mapped KCNQ1. The strongest new association signal was found for SNP#1 located 2.6 kb upstream of a gene involved in pancreatic islet development (P=5.46x10^{-6}). The second strongest signal was found with SNP#2 (P=8.28x10^{-6}), which lies 24.5kb downstream from a gene involved in the conversion of ketone bodies. Our study may lead to a better understanding of differences in the molecular pathogenesis of T2D among various populations.

P09.234
Visfatin gene mutation may increase tendency to nephropathy among diabetics

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Visfatin is a novel adipocytokine, which is suggested to play a role in kidney diseases. This study was performed to evaluate the association between visfatin gene promoter region SNPs and diabetic nephropathy. SNPs (1001T/G, 423A/G, 1535C/T) of the visfatin gene promoter region were studied for 30 subjects with diabetic nephropathy, 30 subjects without nephropathy and 30 healthy subjects, who served as controls, by real-time PCR method. Routine biochemical parameters, serum insulin, TNF-α, visfatin, urinary protein and microalbumine were tested in subjects. Insulin resistance was evaluated by HOMA method. Heterozygotes for the SNPs 423 A/G and 1001 T/G had significantly more risk to have nephropathy. They had lower serum visfatin levels than the subjects with AA and TT genotypes. There wasn't any relation between serum visfatin levels and the serum insulin, TNF-α, BMI, HbA1c, insulin resistance, proteinuria and creatinine clearence. But, healthy volunteers and diabetics with nephropathy had significantly lower serum visfatin levels compared to the diabetics without nephropathy. The two SNPs, 1001 T/G and 423 A/G were in perfect linkage disequilibrium.

To the best of our knowledge, this is the first study indicating an association between diabetic nephropathy and visfatin gene polymorphism. High visfatin levels may harm the kidney and after development of nephropathy visfatin levels may decrease.

P09.235
Meta-analysis of genome wide association studies reveals new loci associated with childhood obesity

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Genetic determinants of adult obesity have been established through meta-analyses of genome wide association studies (GWAS). However, less progress has been made to establish genetic influences specific to childhood obesity though similar approaches. To identify novel genetic factors that influence early-onset obesity, we performed a meta-analysis of genome-wide genotyped datasets consisting of 5,447 cases (>=95th percentile of BMI achieved any time from age 2 to 18 years old) and 8,185 controls (<50th percentile of BMI consistent throughout all measures during childhood) of European ancestry. Following the meta-analysis of ~2.54 million SNPs (directly genotyped or imputed), variation at seven loci showed notable evidence for association with childhood obesity (P < 5x10-8). All these loci have been previously reported in the context of adult BMI GWAS (FTO, MC4R, TMEM18, POMC, FAIM2, TNNI3K and SEC16B), but their relative magnitude of association were different in the childhood obesity setting.TMEM18 gave the strongest evidence for association while TNNI3K and POMC, which were only detected in adult studies when using hundreds of thousands of participants, were readily detected in our relatively small sample size. We elected to take forward all novel loci yielding association with P < 5x10-6 (n = 11) in order to test for replication in multiple existing datasets. To date, we observe consistent evidence for replication at two loci, harboring the genes encoding alpha-protein kinase 1 (ALPK1) on 4q25 and glypican 5 (GPC5) on 13q31, respectively. As more data for the replication attempt is received, we anticipate additional signals will survive this effort.

J09.01
PON1 phenotype and gene polymorphisms in children with autism

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Autism is a neurodevelopmental disorder of unknown etiology. Many genetic and environmental factors are incriminated in its pathogenesis. Subacute exposure to organophosphates (OPs) during critical periods of prenatal neurodevelopment is one of the factors that may trigger autism. Human serum paraoxonase (PON1) is a HDL-associated hydrolase, which in addition to hydrolyzing the OPs, plays a physiological role in reducing LDL oxidation. The PON1 gene shows two functional polymorphisms: M55L and Q192R. These polymorphisms influence the concentration and activity of PON1 towards different substrates, including OPs.

The gene-environment interaction is tested in the present study by assessing the distribution and frequency of M55L and Q192R polymorphisms in 58 autistic children and 43 controls.

No significant difference (p>0.05) in genotypes distribution was found between autistic patients and controls (QQ 51.72%, QR 43.1%, RR 5.17% / MM 12.1%, ML 60.3%, LL 27.6% for autistic group and QQ 51.16%, QR 44.18%, RR 4.65% / MM 9.3%, ML 53.5%, LL 37.2% for control group).

Although, the paraoxonase activity did not present a significant difference between the patients and controls (p>0.05), there was a significant activity increase in the autistic groups with R allele number and both in autistic and control groups with L allele number (p<0.05). The arylesterase activity was significantly decreased (p<0.05) in autistic patients, with no relation to the number of R alleles, but increased in relation with L allele number (p<0.05).

These results provide support for the hypothesis that PON1 arylesterase activity could represent a biochemical/genetic test in estimating autism risk.

J09.02
Assessment of functional impact in lumbar disk disease

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Lumbar disk disease is a common musculoskeletal disease that has a very strong genetic component, revealed by elevated risks to both near and distant relatives supporting a heritable contribution to the development of the disorder. The aim of this study was to correlate some functional indicators that are used for patients with lumbar disk disease. The lot was formed by 103 cases with clinical diagnosis of low back pain accompanied or not by radiculopathy through lumbar disk hernia, examined in the Medical Rehabilitation Clinical Hospital Felix Spa, between February-November 2010. All patients were investigated by MRI, which confirmed the clinical diagnosis. In order to evidentiate the impact of the lesion on functionality and daily activities, we used pain appreciation and consecutive disability parameters: Oswestry questionnaire. We also used MOS SF-36 Health Survay. Using AMA Guide criteria we have evaluated infirmity that generates disability, measuring mobility of dorso-lumbar column. Correlation between disability, investigated with Oswestry questionnaire, a functional evaluation scale, and infirmity evaluated through AMA Guide criteria, mobility evaluation parameter, was small, but significant (p=0.025).

Life quality indicators registered with MOS SF36 and participation restriction evaluated with AMA, strongly correlate, statistically very significantly (p<0.00001). The strongest correlation was noticed between participation restriction revealed by AMA and variable "physical function" of MOS- SF36 scale (r=0.49) statistically very significant (p<0.00001). Affection of mobility had a moderate functional impact on the organism as a whole, but evaluation of life quality of patients revealed that it decreased to half of the standard value.

J09.03

Comparison of DRD1-DRD5 Expression Profiles in Rheumatoid arthritis and Systemic Lupus Erythematous Patients

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Background and Aims: Rheumatoid arthritis (RA) and Systemic lupus erythematosus (SLE) are both autoimmune disease that their causes are still unknown. It appears that both genetic and environmental factors play a role in their pathogenesis. Dopamine and its receptors are the important components of neurotransmission network. Recent studies reveal that in addition to the CNS, immune cells synthesis neurotransmitters such as dopamines so that these catecholamines can regulate immune functions. The aim of this study is to evalute the dopamine receptor gene expression level on peripheral blood mononuclear cells of RA and SLE patients compared to normal individuals.

Material and Methods: In the present study, we investigated dopamine receptor gene expression in PBMCs of 30 RA and SLE patients and 20 healthy individuals using Real Time-PCR. The specificities of the obtained Real time PCR products for the respective dopamine receptors fragments were confirmed by sequenced analysis capillary system (ABI3700, Applied Bio system, USA).

Results: The results showed that D2 and D4 receptors mRNA Expression significantly altered in PBMC of RA and SLE patients. Compared to normal individuals, D2 receptor mRNA in RA and SLE patients

decreased, whereas D4 receptor mRNA Expression increased. The changes of D1, D3 and D5 receptors mRNA Expression was not significant.

Conclusion: It is suggested that dopamine and its receptors have an effective role in pathophysiology of RA and SLE diseases.

J09.04

Identification of Novel Mitochondrial Homoplasmic T14512C Mutation in Iranian Brugada Syndrome Patients

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Brugada syndrome is a disorder characterized by sudden death associated with one of several ECG patterns characterized by incomplete right bundle branch block and ST elevations in the anterior precordial leads. We identified a homoplasmic T14512C mutation in the mitochondrial ND6 gene in 20 unrelated patients with Brugada syndrome patients by PCR-SSCP. This Mutation causes a change of Met to Val (M54V missense mutation). In this study, the effects of this missense mutation upon transmembrane helixes were assayed by means of PolyPhen database (Polymorphism Phenotyping). **PolyPhen** is an automatic tool for prediction of possible impact of an amino acid substitution on the structure and function of a human protein. The result of this prediction showed that M54V mutation is benign. Thus, further investigations necessary to clarify this correlation.

J09.05

Angiotensin-converting enzyme gene I/D polymorphism and ischemic stroke in Moldavian patients

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The aim of the present study was to examine the possible role of D allele as a risk factor in the development of ischemic stroke. It was analyzed 156 patients with ischemic stroke and 90 controls without cardio-vascular diseases.

The frequency of D allele was 63.8 % in patients and 43.3% in controls (x^2=1.76, p=0.19). The distribution of the ACE gene genotypes frequencies weren't statistically significant between stroke group and controls (x^2=2.70, p=0.26).

But a significant increase in the frequency of ACE DD genotypes has been found in studied elderly male patients with ischemic stroke (OR=3.53; CI, 1.05-11.86, x^2 =4.90, p=0.03) when compared ACE genotypes frequencies in the male of control group.

No increase in ACE DD genotype frequency was seen in all females when compared to the respective control group (x^2=0.81, p=0.67).

Also we have reported the D allele (60%) in hypertensive group of male carriers is more prevalent compared with female patients (50%) and male carriers of control group (48.4%) (x^2=0.3.62, p=0.04). There is significant difference in the correlation of D allele carrier and patient serum total cholesterol, triglyceride with correlation respectively coefficients r = 0.25 (p = 0.03) and r = 0.36 (p = 0.02).

In this study we have demonstrated that DD genotype have some association with hypertensive male individuals, the data shown statistical significance. The ACE D allele in male could be is an independent risk factor of development of the hypertension and ischemic stroke in elderly persons.

J09.06

Thrombosis in young men homozygous for Leiden mutation

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Hereditary thrombophilia is one of the most important medical and social problems. It is characterized by activation of coagulation system and lead to the propensity to develop thrombosis. It is known that factor V Leiden is one of the main members of coagulation pathway. Factor V Leiden thrombophilia is characterized by a poor anticoagulant response to activated protein C and an increased risk for venous thromboembolism. Mutation G1691A (rs6025) of *F5* gene also called as Leiden mutation is responsible for such condition. In European countries allele 1691A frequency is around 2%. Homozygous genotype for Leiden mutation is very rear.

We report three cases of thrombosis in young male patients homozygous for Leiden mutation. Beside Leiden mutation in *F5* all three patients were genotyped for SNPs in *F2* (G20210A or rs1799963), *MTHFR* (C677T or rs1801133 and A1298C or rs1801131), *MTRR* (A66G or rs1801394), and *MTR* (A2756G or rs1805087). Data on patients is shown in the table 1.

It is important to mention that in all three cases additional exogenous factors as smoking and long hiking together with exposure to cold were presented. Thus thrombosis can occur in very young patients with combination of genetic risk factors of thrombophilia. It is important to detect such predisposition as early as possible to be able to prevent thrombotic complications.

Characteristic of patients (DVT - deep vein thrombosis)

	Genotype							
	F5	F2	MTHFR	MTHFR	MTRR	MTR		
Patients	G1691A	G20210A	C677T	A1298C	A66G	A2756G	Additional risk factors	Age of first DVT
#1	A/A	G/G	T/T	A/A	A/A	A/G	Smoking, hyperhomo-cisteinemia	18
#2	A/A	G/G	T/T	A/C	G/G	A/G	Long hiking, exposure to cold	19
#3	A/A	G/G	C/T	A/A	A/G	A/G	Smoking	26

J09.07
Molecular genetics of severe asthma in children: no association with CFTR gene mutations common to Russian populations

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Background: Currently little is known from the literature about a relationship between severe asthma (SA) in children and cystic fibrosis (CF). We hypothesized that children with severe asthma may carry a «mild» and/or a «severe» mutations of cystic fibrosis transmembrane conductance regulator gene. Objective: To detect sixteen CFTR gene mutations most common to Russian populations in children with severe asthma. Patients and methods: SA group included 59 children aged 4 - 17 years old (43 boys and 16 girls) with severe asthma. CF group included 29 children aged 5 - 16 years old with a primary diagnosis of CF confirmed by CFTR genotyping (15 boys and 14 girls). Genotyping of CFTR gene was confirmed by experts Laboratory Ott's Institute of Obstetrics & Gynecology. We used two kits developed by Center for Molecular Genetics (Moscow): "CF - 5" kit (G542X, W1282X, N1303K, 3849+10kbC>T, R334W) and "CF - 11" kit (del21 kb, F508del, I507del, 1677delTA, 2143delT, 2184insA, 394delTT, 3821delT, L138ins, 604insA, 3944delTG). Results: In the group of children with CF, the frequency a major mutation F508del was 79% (48% with genotype F508del/F508del and 31% with compound genotype F508del/others). In 14% of the cases, there were identified some other mutations of the CFTR gene: N1303K, 394delTT, 2143delT, CFTRdele2, 3 (21kb). The rest 7% of the cases were not clarified. We have found neither «mild» nor «severe» the mutations of CFTR gene in the SA group. Conclusion: This study failed to show an association of mutations of CFTR gene with severe asthma in children.

J09.08
What a role vitamin D receptor and its gene polymorphism can play in the development of axial myopia in children?

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The axial myopia in children caused by the eyeball overgrowth in the anteroposterior direction is an actual problem of pediatric ophthalmology today. The aim of this study was to investigate a probable association of TaqI polymorphism of vitamin D receptor (VDR) gene with the development and severity of axial myopia in children to identify its possible role and the role of its protein product in the eye tissues development. We examined 84 children (168 eyes) aged 4 to 17 yo with emmetropia and axial myopia of various degree. The results of echo biometric evaluation of the axial length, anterior chamber depth (ACD), lens thickness, and vitreous chamber depth of each eye were compared according to the TaqI genotypes polymorphism detected by PCR-RFLP method. We have found a relationship between the TaqI polymorphism of the VDR gene and the size of the ACD that correlated with the axial length of the eyeball. ACD for tt-genotype carriers was 3.73 Â± 0.04 mm, and ACD for b/T-allele carriers was 3.44 Â± 0.05 mm, ($p < 0,001$). TaqI polymorphism affects the vitamin D-binding domain of the receptor. Therefore we hypothesize that VDR gene polymorphism in combination with external factors such as vitamin D and/or ultraviolet deficiency might be a cause of axial myopia in children. Undoubtedly, further studies addressed to the identification of a role of vitamin D receptor and its gene, as well as an implication of vitamin D and ultraviolet exposures, in the development of axial myopia in children are required.

J09.09
No association between functional polymorphisms in MTHFR and childhood schizophrenia in Latvian population

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Introduction Methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine re-methylation to methionine. Previously it has been reported, that there is association between MTHFR mutations C677T and A1298C, and increased risk of schizophrenia, there are also publications without any association.

The aim to detect frequency of *MTHFR* polymorphisms C677T and A1298C in children with schizophrenia, and to compare results with control group

Materials and methods The patient group was 53 children with schizophrenia, but the control group consisted of 150 volunteers. DNA was extracted from whole blood and purified by standard phenol/chloroform protocol. The presence of C677T and A1298C mutations was analyzed using PCR with subsequent restriction enzyme *Hinf I* and *MboII* digestion, respectively, and detected in PAGE.

Results The observed frequency of the T allele of the C677T mutation was 0,324. There were no statistically significant differences between patient group and control group- 0,319 (p value 1,0). The observed frequency of the C allele of the A1298C in patients group was 0, 311 whereas in control group - 0,367, the difference was not statistically significant (p value 0,395).

Distribution between genotypes in patients' groups is showed in table

	Wild type (C677T)	Wt/C677T	C677T/C677T
Wild type (A1298C)	6	16	5
Wt/A1298C	12	6	1
A1298C/A1298C	6	1	0

Conclusions

1. The present results suggest that the investigated MTHFR polymorphisms do not influence susceptibility of schizophrenia

2. It is necessary to continue this study with a greater number of patients.

J09.10
The study of a role of genes catechol-O-methyltransferase (Val108Met), tyrosine hydroxylase *TH* (VNTR in 1 exon), and a dopamine receptor DRD4 (VNTR in 5 ' UTR '-areas) in development of disease and neuropsychological abnormalities at Parkinson's disease patients

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The purpose of the study is searching of the genetic factors and neuropsychological abnormalities in Parkinson's disease (PD) development. During our clinic and genetic research of Parkinson's disease we've examined PD patients (N=658) and control individuals (N=538). Psychological investigation of 319 PD was carried out. In the expanded samples of patients and control individuals the association analysis of PD with polymorphic variants of monoamine metabolizing enzyme genes was performed: *TH* (VNTR in 1 exon), COMT (Val108Met) and DRD4 (VNTR in 5 ' UTR '-areas). The most important results have been received in *COMT* gene analysis, giving evidence concerning the influence of Val108Met polymorphism on PD development: genotype *H/*H and allele *H, determining synthesis of the enzyme with high activity, may be considered as genetic markers of the increased risk contributing to development of PD in Bashkortostan Republic as a whole and to the most severe PD form, in particular. Genotype *H/*H and allele *H were also risk markers for the development of such neuropsychological abnormalities as personal anxiety, depression, dementia, whereas the genotype *L/*H, defining average activity of *COMT*, made protective impact, reducing risk of PD development. The comparative analysis of the investigated polymorphic loci of TH and DRD4 genes hasn't revealed significant differences between PD samples and controls.

J09.11
eNOS polymorphism and ACE polymorphism gene in secondary arterial hypertension in a romanian children population

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The aim of our study is to investigate whether genetic polymorphisms in the endothelial nitric oxide synthase (eNOS) gene (in intron 4 (4b/4a)) or insertion/deletion (I/D) polymorphism of the angiotensin-1 converting enzyme (ACE) gene are associated with secondary hypertension (HT) in children.

We genotyped 40 healthy (controls), and 38 hypertensive children and adolescents. We compared the distribution of eNOS and ACE genotypes in the two study groups of subjects. Arterial hypertension was defined as systolic/diastolic blood pressure measurements higher than 95 age-gender-height percentile of the adopted reference values. The 4a/a and 4a/b genotype for the intron 4 polymorphism was more common in normotensive than in hypertensive children. The 4a/b eNOS genotype distribution in patients with secondary hypertension (aa= 0%, ab= 23,68%, bb= 76,32%) did slightly differ from genotype distribution in controls (aa= 5%, ab= 37,5%, bb= 57,5%), and the bb genotype was not associated with secondary hypertension (RR 1.611; CI (0.9-2.88); p = 0.09). Moreover, the I/D ACE genotype distribution in patients with HT (DD= 18,42%, ID= 68,42%, II= 13,16%) did differ significantly (p=0.029) from genotype distribution in controls (DD= 47,5%, ID= 42,5%, II= 10%), and the DD genotype was not associated with HT

(RR 0.4516; 95% CI 0.23-0.88; P = 0.0084). In conclusion, we failed to demonstrate that the 4a/b eNOS polymorphism and the ACE I/D polymorphism are genetic markers for secondary hypertension in children.

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J09.12

ACE gene polymorphism in children with nephrotic syndrome in a Romanian population

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Nephrotic syndrome (NS) is one of the commonest renal problems encountered in children. The role of angiotensin-converting enzyme (ACE) insertion/deletion (I/D) gene polymorphism in various renal disorders has been investigated. We have evaluated the frequency of ACE polymorphisms and their impact on the clinical course of INS in children in a romanian county hospital.

This study comprised 20 pediatric patients with nephrotic syndrome, 11 males (55%) and 9 females (45%). Forty children without previous renal diseases and negative proteinuria were enrolled as a control group (50% males and 50% females). Polymerase chain reaction amplification was performed on genomic DNA isolated from peripheric blood leucocytes.

The distribution of ACE DD, ID, and II genotypes in NS patients were 20; 70 and 10%, respectively; the corresponding numbers for the control group were 47,5; 42,5 and 10%, respectively. Angiotensin-converting enzyme genotypes were no significantly different between patients and control groups (p>0.05).

The study groups consisted of 14 (70%) with steroid-sensitive nephrotic syndrome (SNSS) and 6 (30%) with steroid-resistant nephrotic syndrome (SRNS). The distribution of the ACE genotype was II, 14,29%; ID, 64,29%; and DD, 21,42% in the SSNS population and ID, 83,33% and DD, 16,67% in the SRNS population. No statistically significant difference was found between steroid sensitivity and ACE genotypes.

Conclusion: The current study reveals no association between the ACE gene I/D polymorphism and clinical course and steroid responsiveness. Further studies with a larger number of patients are needed.

J09.13

A/G *CKMM* and C/T *AMPD1* gene polymorphisms in elite Russian athletes and response to endurance training

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There is evidence that athletic performance is influenced by genetic factor. Skeletal muscle isoforms of creatine kinase and AMP-deaminase are important regulators of muscle energy metabolism during exercise. There is a conflicting data concerning whether A/G *CKMM*(rs8111989) and C/T *AMPD1*(rs17602729) gene polymorphisms are associated with endurance performance related phenotypes.

Purpose of the study was to determine whether there is a difference in A/G *CKMM* and C/T *AMPD1* genotypes distributions between elite Russian endurance athletes (EA) and students of Saint-Petersburg Universities without training experience (Con), and to investigate the association of these polymorphisms with VO_{2max} and its response ($deltaVO_{2max}$) to 4 months endurance training program.

The analysis was performed on DNA of 170 EA and 209 Con. VO_{2max} was measured for EA during cycle ergometry tests, for 27 EA - before and after 4 months of endurance training. The A/G *CKMM* and C/T *AMPD1* gene polymorphisms were detected by RFLP-analysis.

There were no differences in genotypic and allelic distributions of A/G *CKMM* SNPs between EA and Con. Frequencies of *AMPD1* CC genotype and C allele were significantly (P<0.01) higher for EA compared with Con. VO_{2max} of EA was genotype-dependent for *CKMM* (AA:59.9±8.2ml/min/kg, AG:54.4±9.3ml/min/kg, GG:43.2±9.2ml/min/kg,P<0.001) and *AMPD1* (CC:54.8±7.9ml/min/kg, CT+TT:47.6±5.4ml/min/kg,P<0.001). A significantly lower mean $deltaVO_{2max}$ was detected in EA with GG genotype (AA+AG:15.5ml/min/kg, GG:8.5ml/min/kg,P<0.04) and in T *AMPD1* allele carriers (CC:15.0ml/min/kg, CT+TT:9.2ml/min/kg,P<0.03).

Results provide evidence that A/G *CKMM*(rs8111989) and C/T *AMPD1*(rs17602729) gene polymorphisms contribute to differences in VO_{2max} and VO_{2max}-response to endurance training and might be molecular markers for endurance performance in athletes.

P10 Evolutionary and population genetics, and Genetic epidemiology

P10.01

ADAM33 polymorphisms and overall, cardiovascular and COPD mortality: prospective cohort study

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Background: The *ADAM33* gene is associated with the pathophysiology of COPD and atherosclerosis. This study investigated overall, cardiovascular (CVD) and COPD mortality, in relation to SNPs in *ADAM33*.

Methods: The vital status of 8,465 participants in the Vlagtwedde/Vlaardingen cohort study was assessed at December 31st, 2008. Vlagtwedde/Vlaardingen is a general population based cohort with a follow-up of 25 years (1965-90) with medical surveys every 3 years. We genotyped four *ADAM33* SNPs (S_1, S_2, T_2, Q_1) in 1,390 subjects participating in the last survey. We performed Cox regression to estimate the risk of the SNPs in relation to mortality, with adjustment for gender, age, FEV_1, height, place of residence and packyears of smoking. In addition, stratified analyses according to gender and smoking habits were performed.

Results: At December 31st, 2008, 284 (20.4%) of the included subjects had died (107 due to CVD and 20 due to COPD). Homozygous mutants for SNP T_2 had an increased risk of total and CVD mortality compared to wild types [HR=3.6 (95% CI 2.0- 6.7) and 3.4 (1.2-9.5) respectively]. Individuals homozygous for the minor allele of S_1, S_2, T_2 or Q_1 had a significantly increased risk of COPD mortality. In stratified analyses the risk of total mortality associated with SNP T_2 did not change: females [3.5 (1.5-8.3)], males [3.1 (1.2-7.6)], never smokers [3.8 (0.9-16.3)], ever smokers [3.6 (1.8-7.2)]. **Conclusion:** This study shows for the first time that *ADAM33* is a pleiotropic gene that is associated with total, CVD and COPD mortality, independent of potential confounders.

P10.02
A statistical method for estimation of the age of an admixed population

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Whereas the vast majority of DNA sequence-level variation evolves neutrally and is maintained by a balance between the mutation process and random drift, some parts of the human genome have been subjected to natural selection. Admixed populations are unique in that they represent the sudden confluence of geographically diverged genomes with novel environmental challenges. Thus, genomes from each ancestral population were presented with new challenges. This kind of selection pressure may be quite different from that faced by stationary populations, in which the local environmental changes may occur gradually, allowing for rare advantageous alleles to increase in frequency. Admixed populations therefore offer special opportunities for studying recent selection.

In this study, we propose a method for estimating the 'age' of an admixed population. Using Kimura's (1955) solution of a process of random genetic drift, we developed a novel method of determining the time of a past admixture event based on genetic data comprising of both correlated and independent markers. An extension of the work has been identification of markers exposed to natural selection followed by proposition of a revised time estimator for the admixture event.

Using simulated datasets generated under different assumptions, we have shown that the estimators we propose are robust to fluctuations in the time of the admixture event, proportion of mixing and the genetic divergence between the ancestral populations. However increase in population size and number of markers attributed to a remarkable improvement in the efficiency of the estimators, even under the scenario of natural selection.

P10.03
Interactions between genetic ancestry and socioeconomic status shape self-identification and discrimination among Puerto Ricans

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Puerto Ricans are a complex society resulting from the admixture of the original Taíno Amerindians, Europeans, and Africans. This admixture process was not random and we identified assortative mating based on genetic ancestry in a previous study. Moreover, historical processes have contributed to the geographic heterogeneity in the distribution of admixture across the island of Puerto Rico, which also influenced social differences.

Here, we analyze a census-based sample of 405 Puerto Ricans that fulfilled a comprehensive survey about the concept of race, the use of racial classifications, perception of discrimination, and social class. These individuals were also genotyped using a panel of 106 Ancestry Informative Markers (AIMs) to determine their admixture proportions. Our sample of Puerto Rican individuals showed average proportions of 19.5% African, 64.3% European, and 14.9% Amerindian ancestries, similar to previous studies. There were significant differences in ancestry between individuals that self-identified as "White" or "Black", although both groups overlapped in the distribution of ancestry values. Other variables, such as socioeconomic status, income, or education, contributed to the differences in self-identification and to differences in the perception of race and discrimination. As previously observed for other demographic factors, Amerindian ancestry did not play any substantial role in these processes. These results have substantial implications to understand the social and demographic processes that have formed this population.

P10.04
Grading the credibility of genetic associations in Alzheimer's disease using the Interim Venice Criteria

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The Alzgene database is a valuable resource for researchers working in Alzheimer's disease (AD). In the database, the credibility of genetic associations is graded using the interim Venice Criteria but these criteria have never been formally evaluated. We evaluated the robustness of the Venice grades in Alzgene data in a simulation studies by adding hypothetical results of simulated studies to the meta-analyses in the top list. We further conducted a follow-up study of the interim grades of the top list. The Alzgene 'top list' changed considerably (40%) over the course of one year. A total of 3 genes with strong credibility were graded to weak within this short period. When evaluating the question whether this is due to a high type 1 error (p-values) or type two error (low power), we found that increasing power had very little effect on the positive predictive value of a finding, but the impact of testing with a lower p-value is major. Based on a data-freeze of December 2009 our simulations show that many genes could drop due to a low OR (<1.15; bias criterion), except for APOE and ACE. .Our simulation studies show it is impossible to change the credibility of ACE based on the heterogeneity (I2). However, the credibility for ACE altered empirically due to first study bias. Based on our finding we propose evidence criterion by a criterion based on the p-values and to replace the low summary OR criterion for bias by a criterion based on the presence of replication.

P10.05
Variation at Follicle Stimulating Hormone Receptor (FSHR) gene and age at natural menopause influence the onset of Alzheimer's disease in women.

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There is evidence of a higher prevalence of Alzheimer's disease (AD) in women, and it is becoming clear that it may be due not only to their longer life expectancy, but also to biological risk factors. In previous investigations we observed that, in addition to genes involved in estrogen metabolism (ESR1 and CYP19), also gender-specific factors such as past fertility may play a relevant role in the development of AD in women. In the present study we investigated the possible influence on AD onset of FSHR gene, that is involved in the regulation of fertility in women. As a possible AD risk factor, age at natural menopause was examined as well.

In a sample of 212 women with late-onset sporadic AD and 85 control women the FSHR Thr307Ala (rs 6165) polymorphism was examined. A significant excess of FSHR GG genotype (Ala/Ala) (OR = 0.46 , 95% CI 0.24-0.90, p=0.02) was observed in controls suggesting that Ala/Ala genotype could have a protective effect on AD development in women. AD women had an age at natural menopause (49.7± 0.21) lower than controls (50.6± 0.34, p=0.02) and linear regression analysis showed a significant positive relationship (p=0.03) between age at natural menopause and age at AD onset. These observations seem to support the hypothesis that the loss of neuroprotective estrogens after menopause may play a role in AD development in women.

P10.06
Association of genetic polymorphism at the APOAI/CIII/IV/V gene loci with variation in plasma lipid levels among a sample of the general Kuwaiti population

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Apolipoproteins are responsible for the clearance of lipoproteins, failure of which may lead to the development of dyslipidemia. Numerous single nucleotide polymorphisms (SNPs) have been identified at the APOAI/CIII/IV/V gene cluster on chromosome 11q23-24 that may alter the transport and/or metabolism of lipoproteins. As hypercholesterolemia and hypertriglyceridemia have been reported to be highly prevalent in the Kuwait population, the objective of this study was to determine genetic polymorphisms at the APOAI/CIII/IV/V

locus in a sample of the general Kuwaiti population (n=482). A total of 8 SNPs were genotyped and analyzed with regards to plasma lipid levels, BMI and Arab ethnicity. The SNPs included APOAIV Q360H and T347, APOCIII 3238C with APOCIII 3238C›G, -455T›G, -484C›T as well as the APOAV -1131T›C, c.56c›G and c.1259T. Genotype and allele frequencies were determined by simple gene counting methods and tested for Hardy-Weinberg Equilibrium and Linkage disequilibrium using GENEPOP (Version 3.4) while haplotypes were constructed using HAPLOVIEW. Genotype and allele frequencies as well as haplotypes were comparable to those reported in other populations and will be presented. Multivariate analysis was carried out to investigate the association of minor alleles with variation in lipid levels. Preliminary results indicated a genetic component to be involved in predisposing Kuwaiti's to dyslipidemia. Investigating potential direct or indirect involvement of minor alleles in Kuwaiti's could reflect the interaction of environmental factors such as fat-rich diet and sedentary life-style with the diverse ethnicity in Kuwait to promote dyslipidemia. Further analysis of other polymorphisms in a larger sample is currently being analyzed.

P10.07
Association of the APOD gene with cardiovascular and metabolic events

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ApoD is a lipocalin which plays a role for transport of hydrophobic molecules. While it is well known that ApoD has tissue-specific physiogical ligands and functions, these are still subject for exploration. Transgenic mice over-expressing ApoD show changes in lipid and glucose metabolism.

Our previous findings show that ApoD is present among genes that are expressed mainly in the heart and its expression is significantly decreased on atherosclerotic plaques. Furthermore, as a preliminary finding, variations at the functional regions of ApoD gene on 50 patients with coronary heart disease has been explored using DHPLC and differences in sequences were found on 16 patients. These pilot studies lead us to the hypothesis that ApoD gene polymorphisms and protein may play a role in cardiovascular diseases and metabolic syndrome in humans. We further analyzed one of those SNPs in in our DNA bank of 2235 people specific for Turkish Population, There were significant associations with this genotype and hypertension, physical activity in the whole group. Gender-specific analyses showed that there was an association with ApoA1 levels and physical activity after exclusion of drug usage in women. In men, however, hypertension, physical activity as well as fasting glucose levels, after exclusion of drug usage, were found to be associated.

These is the first study to show that ApoD might play a role in cardiovascular and metabolic event. ApoD-protein analyses can be performed and investigated, if this protein can be specified as a biomarker.

P10.08
Autosomal recessive disorders in the South Mediterranean Region: An overview from North Africa

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Hereditary diseases and congenital abnormalities have been reported to affect 2 - 5 % of all live births. Actual data suggest that genetics disorders are common also in countries of the North Africa. This population is made of a mosaic of communities. This heterogeneity with the same social and economic structure has contributed to increase the rate of endogamy and consanguineous unions. These practices lead to the emergence of autosomal recessive.

Aim: The aim of this study was to report the genetics spectrum of autosomal recessive disorders and analysed their prevalence in North Africa.

Methods: An exhaustive electronic and library research of the recent publications was carried on the biomedical databases Pubmed and OMIM using Keywords "autosomal recessive diseases", "North African patients", "Founder effect".

Results: The distribution of the autosomal recessive disorders was not uniform and some traits are common. Some examples are: Familial Mediterranean Fever, ßthalassemias, cystic fibrosis. The relative homogeneity of populations from North Africa has often been underlined, and several founder mutations have already been suggested. A number of rare diseases described from North Africa have now been few recognised in other countries; this result suggests that the private syndrome does exist such as Andermann Syndrome, Hutchinston-Gilford syndrome and Anderson disease. For theses reasons, our region will continue to be a source of new information's about genetic disorders for the whole world. With the special religious and cultural backgrounds, more work should be given in planning and research ways of prevention and treatment of genetic disorders.

P10.09
STR data from forensics: what can it tell us about human population history?

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Forensic genetics is prolific in generating genetic profiles based on Short Tandem Repeats (STRs), at a worldwide scale. The most used commercial kits include 13 common autosomal STR loci, being widely screened for consistency and quality control purposes between labs. The availability of such amount of data at a worldwide scale drove us to construct an online database of allele frequency data - Strdna-db - and an online tool comprising genotype profiles - PopAffiliator. Here, we intended to understand how informative these markers used in forensics can be to unravel human demographic history. The analysed datasets included frequency data - 190 populations and 69,229 individuals - and genotype data - 43 populations and 11,181 individuals.

Inter-population comparisons indicate a significant level of population structure, with a global FST value of 4.3-5.0% for both datasets, thus in agreement with levels inferred in non-forensic studies. This agreement further extends to the pattern of within-population diversity, which shows a decrease from sub-Saharan African to American populations, in accordance with the recent African origin of modern humans. Actually, the geographic distance to East Africa explains 55% of the decrease in genotype diversity, a pattern that could be explained by a series of founder effects during the expansions out-of-Africa.

Our results show that extensive datasets from forensics can be used to address population genetics' questions, and the statistical methods used provide reliable results as they account for the particularities and limitations of those markers.

P10.10
Epidemiological study of Beckwith Wiedemann syndrome in European population

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Beckwith Wiedemann syndrome (BWS) is an overgrowth syndrome characterized by growth disregulation, risk of tumour development and congenital anomalies. In this study we analysed 197 patients registered in EUROCAT network of congenital anomaly registries in 1980-2007 period by monitoring population of 13 546 771 births. The prevalence was 1.45 per 100 000 births. Male to female ratio was 1.3:1. High proportion of patients was diagnosed by prenatal ultrasound examination (70/166; 42.1%), 41 patient was discovered at birth (41/166; 24.69%) and 55 patients (33.3%) were diagnosed during the first month of life. Most patients were live births (177/197;

89.8%), eight were stillbirths (8/166; 4.81%), and twelve pregnancies were terminated after prenatal diagnosis (12/70; 17.4%) due to heart, kidney or limb anomalies. Seven live births didn't survive the first week of life (7/177; 3.9%). In 148 (75.12 %) of patients major malformations were present, with the hallmarks of the syndrome, omphalocele (54.72%; 81/148) and macroglossia (52.7%; 78/148), being the most common. Other associated anomalies were present in 68 (46%) patients: cardiovascular in 20.28 % (30/148), urinary in 17.56% (26/148), limb defects in 9.15% (14/148), and central nervous system malformations and cleft lip in 2.0% (3/148). In thirteen patients more than one congenital anomaly was present. In conclusion, with estimated frequency of 1 in 69930 births, BWS is a rare congenital anomaly disorder, often associated with major congenital anomalies. Pregnancies that are carried out to term mainly result in live births with high survival rate.

P10.11
The relationship between TNF-alpha gene polymorphisms and susceptibility to Behcet's disease in Iran.

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Background- Behçet is a multisystematic disease which includes recurring mouth and genital ulcers along with inflammation inside the eye and skin problems. BD patients have higher serum levels of Tumor necrosis factor (TNF). The positive response of BD patients to Anti- TNF therapy suggests a pivotal role for TNF-alpha in disease expression.

Aim- The aim of the current study was to investigate the possible relation between TNF- alpha-1031T/C and TNF- alpha -308G/A polymorphisms and susceptibility to Behcet's disease in Iranian Azeri Turkish Patients.

Method- In this study, the distribution of two polymorphisms within TNF gene promoter region was compared in 53 BD patients and 79 healthy controls using PCR-RFLP technique.

Findings- A significant difference was observed with respect to the alleles frequency of TNF-alpha-1031C which was higher in BD patients as compared to controls; while the alleles frequency of TNF alpha-308A revealed no difference in the two groups.

Conclusions- The frequency of CG haplo-type was significantly high in BD patients while the frequency of TA haplo-type was significantly low in these patients. The result reveals that in the North West population of Iran TNF alpha gene is involved in susceptibility of Behçet's disease.

P10.12
Genetic basis of Bombay Blood group

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The Bombay Blood phenotype was first discovered in Bombay by Dr.Y.M Bhende, present in about 0.0004% of the world population and in some populations like Mumbai, the occurrance can be as high as 0.01% of inhabitants. This blood group is identified by reverse grouping. The antigens determined by the human ABO blood group locus are oligosaccharide molecules constructed by the sequential action of specific glycosyltransferases, the final step being catalyzed by allelic glycosyltransferases encoded by the ABO locus. These enzymes require an oligosaccharide substrate known as the H blood group antigen, constructed by α-(1,2)-fucosyltransferase; GDP-L-fucose-βD-galactoside 2-α-L-fucosyltransferase, whose expression is determined by H and Secretor (SE) blood group loci known as FUTI and FUT2 genes respectively. Erythrocytes from individuals with rare Bombay and para-Bombay blood group phenotypes are deficient in H determinants, and thus A and B determinants, as a consequence of apparent homozygosity for null alleles at the H locus (h,h) . FUT1 and FUT2 sharing a high degree of sequence homology, are located within a 100 kb region in chromosome 19q13.3 suggesting their common origin. Occurrence of the phenotype among unrelated individuals indicates spontaneous mutations in the gene. A T725G mutation in the coding region of FUT1 and a gene deletion of FUT2 cause classical Indian Bombay phenotype. In para- Bombay blood phenotype (secretor positive), the expression of this variation is due to a missense point mutation at codon 164 in the FUT1 gene. Prior to blood transfusion, people with blood group O should be tested for Bombay phenotype.

P10.13
Genetic-epidemiological study of BRCA1 in an isolated population of South Tyrol. Description of a new mutation.

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Purpose: Due to its geopolitical situation and socio-cultural heritage South Tyrol can be considered a population isolate. The annual incidence of breast cancer in this region is approximately 300 cases. This is the first study investigating the contribution of the cancer susceptibility gene BRCA1 in this population.

Patients and Methods: Patients were identified from the Tumor Registry. Inclusion criteria were a) all breast (BC) and/or ovarian cancer (OC) patients diagnosed in the year 2002, b) BC patients diagnosed age ≤50 between 1998 and 2001 and c) all OC patients diagnosed between 1998 and 2001. DNA was extracted from peripheral blood and direct sequencing of all exons and exon/intron boundaries was performed.

Results: In total 153 patients were analyzed. We identified two deleterious mutations (c.1687C>T; c.4183C>T) in two BC and one OC patients; both of them non-sense mutations leading to a premature stop codon and previously reported in the BIC database. The missense variation c.83T>C was found in one BC/OC patient. We report a novel germline mutation c.5539_5544del6 in exon 24 in a BC patient. This mutation leads to an in-frame deletion of two amino acids in the highly conserved BRCT-domain and to the best of our knowledge has not been documented before. All of the mutation carriers had a positive family history.

Conclusion: The BRCA1 mutation frequency of 3.3% in unselected breast/ovarian cancer patients from South Tyrol is comparable to that of other European populations. To determine if the newly identified mutation is a founder mutation, further analyses are needed.

P10.14
Science of Breeding and Heredity from Ancient Persia to Modern Iran

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The prophet Zoroaster declared equal right for women and men to choose their "own ways". There is much evidence that ancient Persians believed in the equal contribution of women and men towards producing a child, and its hereditary characteristics.

The Greek philosophers believed that man's water (Semen) contains all human characteristics, and the female uterus is only responsible for nurturing and development of fetus.

After detection of the ovum Malpigy proposed the preformation theory (ovist) which means there is a miniature human inside ovum, that grows after Semen has entered the uterus and grow into a well developed fetus. This hypothesis was later delegated to spermatozoa. These contradictory and inappropriate beliefs were subject to dicussions and dispute; until C.E.Wolf demonstrated that the embryo is a product of the fertilization of ovum by spermatozoa.

800 years prior the sage Ferdowsi explains nicely the equal participation of man and woman in the production and transmission of characters.

After the renaissance and especially in recent years tremendous achievements have been made in unraveling biological secrets of reproduction. There was no work on genetics in Iran until 1936; when a genetic course to biology curriculum in related colleges and universities was added Iranian Genetics Society was founded in 1966, initiation steady movement in this field.

Although there was an inevitable gap during the revolution and war, now there is great effort by researchers to eliminate the gap and bring us into the mainstream of world science; development in biomedical sciences in the 3rd Millennium.

P10.15
Detection and genotyping of human papillomaviruses in Bulgarian patients for the period of 2009-2010

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Human papillomavirus (HPV) is now considered as a causative agent of carcinoma of the uterine cervix. Genital human papillomaviruses are classified into low-risk and high-risk groups. The low-risk HPVs are associated with genital warts and lesions, as for the high-risk they are commonly associated with high-grade squamous intraepithelial lesions and cervical cancer. Cervical cancer is the second most frequent cause of death from cancer in women worldwide; however cervical screening programs reduce the incidence of this type of cancer. In this research we have used Roche's LINEAR ARRAY HPV Genotyping Test. This test combines multiplex PCR with 40 pairs of primers with DNA hybridization and analyzes 38 types and subtypes of HPV. The cervical smears were collected from Bulgarian patients with condylata acuminate lesions. One hundred and twenty six cervicovaginal smears and scrapings were tested. Forty two percent of them were found positive for HPV DNA and 58% - negative. We found more then one type of viral DNA in all positive samples. We found in twenty two samples HPV 16 DNA (38%), in eight samples - HPV 18 DNA (18%), in five cases - HPV 6 DNA (10%), in seven cases - HPV 53 DNA (11%), in three cases - HPV 31 DNA (4%), in four cases - HPV 35 (5%) and in two cases we found positive for viral DNA types HPV 61 and HPV 62. Further studies are needed to determine the influence these HPV infections have on the epidemiology of the genital tract of these patients.

P10.16
Upgrading the chimpanzee reference genome

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Comparative genomics is an intensive and fruitful field of research that seeks to unravel the main mechanisms underlying the evolution of the different species. Comparisons between the chimpanzee genome and the human genome have already given huge insights to understand adaptive processes that have taken place after the separation of the two species. In 2005 the first draft genome of a Western chimpanzee (*Pan troglodytes verus*) was assembled using a whole genome shotgun approach using Sanger technology and an effective coverage of 3.6x was reached. However, the draft nature of the assembly precludes deeper analyses, and still the genome contained gaps in the sequence every 15.7 Kbps

We are planning to upgrade the chimpanzee assembly by integrating 640 finished BAC clones, making 49.000 contig joins, improving N50 contig length from 26 kbps to 39 kbps and finishing chromosome 7 entirely in BAC clones. We have also sequenced the same individual at a much higher coverage (40x) using the Illumina technology with read length of 100 bp to help gap closure. We have performed an extensive study of the new assembled chimpanzee genome and we have compared it with the human genome (NCBI 37, hg19). We will put special emphasis to detect and resolve spurious indels or SNP variants, and to identify improperly represented duplicated regions using read depth and paired end mapping methodologies. Moreover, we will reanalyze some of the main statements in the initial study such as a detail characterization of the main differences that separate humans from chimpanzees including indels and structural variation.

We believe that this update will be useful for most of the scientific community working in the genomics field and that by comparing the new assembled chimpanzee genome with the human genome/s, we will improve our understanding of human specific genomic features.

P10.17
Variants in the CYP1A1/CYP1A2 locus are associated with coffee drinking

Variants in the CYP1A1/CYP1A2 locus are associated with coffee drinking

Coffee consumption is a model for addictive behaviour. We performed a meta-analysis of genome-wide association scans (GWAS) on coffee intake from eight Caucasian cohorts (N = 18,176) and sought replication of our top findings in a further 7,929 individuals. Significant evidence of association was detected at a variant (p-*value* = $1.6*10^{-11}$) in the 23-kilobases long commonly shared 5-prime flanking region between *CYP1A1* and *CYP1A2* genes. *CYP1A1* was found to be down-regulated in lymphoblastoid cell lines treated with caffeine. *CYP1A1* is known to metabolize polycyclic aromatic hydrocarbons which are important constituents of coffee, while *CYP1A2* is involved in primary metabolism of caffeine. Significant evidence of association was also detected at another variant (p-*value* = $3.9*10^{-09}$) near the *NRCAM* gene -- a gene implicated in vulnerability to addiction -- in the meta-analysis of discovery and replication cohorts. Our results from GWAS and expression analysis also strongly implicate *CAB39L* in coffee drinking. Moreover, the variant in the *CYP1A1*/ *CYP1A2* locus also showed significant positive association with systolic and diastolic blood pressure.

P10.18
Population variability of SNPs associated with common mental and neurological disorders in North Eurasia

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Ethnic background appears to be an important factor in personalized health care and predictive medicine directed to prevention of common diseases. Common psychiatric and neurological disorders including schizophrenia, Alzheimer's diseases (AD), and Parkinson's disease (PD) are now the subject of intensive genome-wide association studies (GWAS). The aim of this study was to estimate the variability of genetic markers strongly associated with neurological and mental diseases across the multiple Eurasian populations. We selected 55 single nucleotide polymorphisms (SNPs) reported in recent GWAS as highly significantly associated with AD, PD, schizophrenia and cognitive performance, and investigated the population variability of these markers in 8 populations representing Eastern Europe, Central Asia, North Asia and Siberia. Considerable between-population variability in allele frequencies was found. The average range of risk allele frequency for 55 SNPs was 24.3%. Population differentiation measured by Fst varied widely across the loci studied (from 0,006 to 0,146). Mean Fst for all 55 SNPs was 0,045. Mean Fst for AD (0,029) proved to be substantially lower than population differentiation for PD (0,044), schizophrenia (0,054) and cognitive performance (0,049). Allele frequencies tend to correlate between populations according their geographical locations. Our data indicate that wide inter-population variation in the frequency of alleles associated with common diseases exists even on a sub-continental level. This variability may play a substantial role in the variation in genetic predisposition to common neurological and mental disorders in ethnically different populations.

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P10.19
CONAN: Analysis Software for Genome-Wide CNV Association Studie

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Genome-wide association studies (GWAS) based on single nucleotide polymorphisms (SNPs) revolutionized our perception of the genetic regulation of complex traits and diseases. Copy number variations (CNVs) are genomic segments which are duplicated or deleted among different individuals, ranging from kilobases to several megabases in length. CNVs promise to shed additional light on the genetic basis of

monogenic as well as complex diseases and phenotypes. However, while several software packages support the determination of CNVs from SNP chip data, the downstream statistical inference of CNV-phenotype associations is still subject to complicated and inefficient in-house solutions, thus strongly limiting the performance of GWAS based on CNVs.
We present CONAN, a freely available software solution which provides an intuitive graphical user interface for categorizing, analyzing and associating CNVs with phenotypes. CONAN assists the evaluation process by visualizing detected associations via Manhattan plots in order to enable a rapid identification of genome-wide significant CNV regions. Various file formats including the information on CNVs in population samples are supported as input data.
CONAN facilitates the performance of GWAS based on CNVs and the visual analysis of calculated results. CONAN provides a rapid, valid and straightforward software solution to identify a part of the genetic variation underlying the 'missing' heritability for complex traits that remained unexplained by recent GWAS. The freely available software can be downloaded at http://genepi-conan.i-med.ac.at.

P10.20
Identification of *p53* and *CYP2C9* gene polymorphisms among Iranian (Turkmen & non-Turkmen) and Iraqi populations

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The Cytochrome P450 (CYP) 2C9 enzyme is involved in drug metabolism and in detoxification of carcinogenic compounds. It is reported that *CYP2C9* polymorphism is involved in drug resistance. Additionally, the p53 protein is considered a key combination in countering stress messages such as DNA damage. It is reported that *p53* polymorphism at codon 72 is involved in response to chemotherapy. The aim of this study is to determine the prevalence of the *p53* and *CYP2C9* genetic polymorphisms in different ethnic groups in Iran compared with those from Baghdad city in Iraq. After obtaining signed informed consents, blood samples were collected. For this purpose the blood samples were collected from 70 Iraqi healthy individuals and 130 healthy Iranian population (Turkmen and non-Turkmen). We assessed the genotype patterns of *p53* and *CYP2C9* among Iranian ethnic groups. Then, the data was compared with the allele distribution for above genes among Iraqi population. The *p53* genotypes at codon 72 and *CYP2C9* polymorphism at codon 144 were determined by PCR-RFLP analysis and the results were confirmed by DNA sequencing analysis. It is showed that the frequency of Cys allele for *CYP2C9* at codon 144 among Iranian population (10%) was two times higher than Iraqi population (5%). In contrast, Arg allele for p53 gene at codon 72 among Iraqi population (42%) was higher than Iranian population (35%). Thus, these polymorphisms may be a suitable target for research in the field of pharmacogenetics among Iranian and Arab populations.

P10.21
Frequency and origin of 2184insA mutation in CF patients from Ukraine

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Analysis of mutations in *CFTR* gene among CF patients from different populations revealed that highest frequency of 2184insA mutation was observed in Eastern Europe. In our study we screened mutation 2184insA in 351 CF patients from different regions of Ukraine. The frequency of this mutant allele was 5.5%. This is the second most common CF mutation in Ukraine (F508del - apr. 50%).
To determine origin and distribution of this mutation in Ukraine the haplotype analysis of two intragenic polymorphisms (IVS8CA and IVS17bTA) was performed. For haplotype construction of polymorphic loci the carrier parents and CF individual were analyzed. 28 out of 39 chromosomes with 2184insA mutation carried the haplotype 16-7 for IVS8CA and IVS17bTA loci respectively. In 11 families gametic phase could not be established for one or both markers, but in all these cases alleles 16 and 7 were presented in patient genotype.Our results support single origin of chromosomes with 2184insA mutation in our CF population. However, data on haplotype analysis of CF mutations from other studies revealed that 2184insA mutations originated from multiply events - several haplotypes linked to this mutation were observed in different populations. Our results suggest that the appearance of the 2184insA mutation in the gene pool of Ukraine and reaching such high frequency may have been connected with recent founder effect and slippage has not given rise to any microsatellite variability. To clarify this suggestion analysis of additional polymorphic markers will be necessary.

P10.22
Complete screening of CFTR gene mutations in cystic fibrosis patients from Eastern Hungary

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Introduction. Molecular genetic diagnostic strategies of cystic fibrosis (CF) are dependent of the mutation distribution of the analyzed population. The goal of our study was to establish the mutational spectrum of 40 clinically diagnosed severe CF patients in Eastern Hungary.
Materials and methods. For the mutation testing, a multiplex commercially available diagnostic assay was used together with a specific PCR assay for the detection of the CFTRdele2,3(21kb) enabling to detect 30 CFTR mutations. In patients having mutations not included in this panel, the entire coding region of the CFTR gene was sequenced. Large alterations were analyzed using MLPA.
Results. Using the mutation detection kit only, the detection rate was 81%. Integrating the CFTRdele2,3(21kb) mutation to the panel increased the detection rate to 86%. When DNA sequencing was used, disease-causing mutations could be identified in 79 out of the 80 CFTR alleles. The complete analysis verified four 2184insA (5.0%), two L101X (2.5%) and one Q220X, S466X, Y1092X, E831X alleles (1.25% respectively). No large insertion or deletion was found.
Conclusions. Our results suggest, that in Eastern Hungary the majority of CF-causing mutations are small scale. Presence of six mutations (delta F508, CFTRdele2,3(21kb), 2184insA, N1303K, G542X and L101X) was shown in 91% of CF chromosomes. Two mutations (CFTRdele2,3(21kb), 2184insA) were found in suprisingly high frequency, in four patients each (5.0%).

P10.23
Genetic polimorphism of cytochrome P450 genes in the ethnic groups of Bashkortostan

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Aim. To determine the prevalence of the most common allelic variants of CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2C9, CYP2E1, CYP2F1, CYP2J2 and CYP2S1 in a representative sample of the three ethnic population from Republic of Bashkortostan (Russia), and to compare these data with exiting published data from other populations.
Methods. CYPs genotypes were determined in DNA samples of healthy unrelated individuals representatives of three ethnic groups (N=319 Russians, N=279 Tatars, and N=144 Bashkirs. The CYPs genes polymorphisms were examined using PCR-RLFP method.
Results. The frequencies of the variant genotypes, alleles and halpotypes of the CYPs genes were determined. Analysis of the CYP1A1 (rs1048943, rs4646903), CYP1A2 (rs762551), CYP2A6 (rs71790353), CYP2E1 (rs2031920) allele, genotype and haplotype frequencies detected significant differences among healthy residents of Republic of Bashkortostan belonging to different ethnicity. Significant differences among Russians, Tatars, and Bashkirs were not detected for CYP1A2 (rs35694136), CYP1B1 (rs1056836), CYP2F1 (rs11399890), CYP2J2 (rs890293), CYP2S1 (rs34971233, rs338583), CYP2C9 (rs1799853, rs1057910) genes. Analysis of the CYPs genes allele frequency distribution patterns among the ethnic groups of the Republic of Bashkortostan in comparison with the worldwide populations were conducted.

Conclusion. The allele and genotype frequencies distributions of the CYPs genes markers among the inhabitants of the Republic of Bashkortostan demonstrate the influence of different ethnic components on contemporary populations occupying the region of interest. The results of present investigation will form the basis for identification of the genetic risk factors to cancer susceptibility, determining the toxic potentials of environmental pollutants and might the genetic background of drug response.

P10.24
Implementation of a recently developed whole genome amplification method on DNA profiling of old skeletal remains

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An insufficient DNA quantity along with the presence of inhibitors and DNA degradation are of utmost challenges implicated in DNA profiling of old skeletal remains. Apart from approaches using smaller amplicons which have significantly increased the possibility of obtaining DNA profiles from highly degraded DNA samples, amplification of low copy number (LCN) DNA samples and reduction of inhibitory effects are attainable with our recently reported whole genome amplification (WGA) method named KI-PEP PCR. Implementation of this improved degenerate 15-mer primer extension preamplification PCR on the DNA isolated from 44 skeletal remains of Iranian war (1980-1987) victims was followed by specific amplification with specific primers and Real-time PCR. DNA from fresh blood and a mixture of bone and blood DNA samples were also concomitantly examined.
Subsequent to the amplification by KI-PEP PCR, partial DNA profiles were obtained from 15 samples with both Identifiler and minifiler Kits and no superior results were generated in comparison to DNA profiling without prior amplification. These results demonstrated that despite increasing the low quantity of DNA and relieving inhibitors by the utility of KI-PEP PCR, no significant improvement can be achieved in DNA profiling of old skeletal remains on account of the presence of too many degraded DNA and the overall size reduction of DNA by the method.
Keywords: DNA Profiling, Whole genome amplification, Improved primer extension preamplification PCR, Skeletal remains

P10.25
DNA repair gene polymorphisms at XRCC1 (Arg194Trp, Arg280His, and Arg399Gln), XRCC3 (Thr241Met), and XPD (Lys751Gln) in healthy Tunisian population

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Reduction in DNA repair capacity is associated with increased rates of birth defects, cancer, and accelerating ageing. Genetic polymorphisms in DNA repair genes might influence the repair activities of the enzymes predisposing individuals to cancer risk. Owing to the presence of these genetic variants, inter-individual and ethnic differences in DNA repair capacity have been observed in various populations. The present study aimed to determine the allele and genotype frequencies of five non-synonymous SNPs, XRCC1 Arg194Trp (C>T, rs1799782), XRCC1 Arg280His (A>G, rs25489) XRCC1 Arg399Gln (A>G, rs25487), XRCC3 Thr241Met (G>A, rs861539), and XPD Lys751Gln (T>G, rs13181) in Tunisian population and to compare them with HapMap populations. The variant alleles of these SNPs have been found to be positively associated with different forms of cancer in several genetic epidemiological studies in other populations. To the best of our knowledge, this is the first report of these DNA repair gene polymorphisms in Tunisians. The basic prevalence of these polymorphisms in the general population must be known to evaluate their significance in risk assessment in cancer and other phenotypes. DNA was isolated from the peripheral blood sample of 154 healthy and unrelated individuals and the genotypes were determined by PCR-RFLP. The allele and genotype frequency distribution at the five SNPs among Tunisians revealed a characteristic pattern may be explained by the fact that the Tunisian population is a mix of ethnic groups. This could assist in high-risk screening of humans exposed to environmental carcinogens and cancer predisposition in Tunisian population.

P10.26
Down syndrome (DS) space clustering suggests fixed geographical exposures

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Space and time clustering of DS has for a long been a matter of controversy. Recent study suggests clustering to be associated with more densely populated urban areas (McNally et al. Int J Epidemiol 37:1169-1179). St.Petersburg is a large densely populated city with comparatively low migration rate. The study included all cases of DS born alive or prenatally diagnosed in 1970-2009, except for twins, both parentally derived trisomy 21 and trisomy 21 suspected to be parentally derived (recurrent cases in the same family). It was found that 2590 DS cases were unevenly distributed throughout the study area. We have identified 253 cases of a multiple occurrence of unrelated DS at the same dwelling, including 223 clusters of two DS, 23 clusters of three DS, 5 clusters of 4 DS, and two clusters of five DS. Clusters of two DS cases were estimated as a chance coincidence predominantly. Analysis of 30 clusters of 3 to 5 DS showed a highly significant excess over expected figures (one case in 714.6 flats) for 16 of them. Mothers of DS individuals from 16 clusters (n=50) were younger compared to mothers of DS from outside the clusters (n=2409): 28.8 vs. 30.8 yo, proportion of mothers aged 35 and older 20% vs. 35.9%, p=0.02. Only 14% of dwelling units inside 16 clusters were located at the ground floor which makes the effect of radon exposure as a major cause of clustering quite improbable. Our data suggests an effect of some geographically fixed exposures other than radon.

P10.27
Molecular diagnosis of Duchenne muscular dystrophy in romanian patients by MLPA method

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Dystrophinopathies are a group of fatal muscle diseases with X-linked inheritance which have as common cause mutations in the DMD gene. The frequency of the diseases is high - 1:3500 newborns (male). The DMD gene mutations cause in Duchenne muscular dystrophy (DMD) a complete absence of dystrophin synthesis.
Method. 28 patients were analysed and genomic DNA was extracted from blood samples using QIAamp DNA Blood Mini Kit. Samples were collected from DMD patients with clinical diagnosis of DMD, from their relatives and in one case amniotic fluid was collected for prenatal molecular diagnosis. Isolated genomic DNA was analyzed by MLPA technique, which can identify extensive deletions or duplications of gene fragments. The results which were obtained by capillary electrophoresis on AbiPrism 3130XL were quantified and interpreted using Coffalyser program.
Results. In 13 patients were identified extensive deletions (59.1%) and duplications only in two patients (9.1%). 6 female patients with heterozygous genotypes confirmed the previously identified mutations in male patients. In one case prenatal diagnosis was performed on a patient having a child with DMD, confirmed by molecular diagnosis. In 6 patients (27.27%) no deletions or duplications were identified.
Conclusions
Our results confirm literature data that indicate a higher frequency of large deletions and a lower frequency of duplications, the two types together representing over 68% of mutations identified in patients with clinical diagnosis of DMD. MLPA technique is particularly effective if

Duchenne disease, allowing the detection of mutations in most cases and both prenatal diagnosis and diagnosis.

P10.28
FMR1 haplotype analysis in mentally retarded male population

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DXS548-FRAXAC1-FRAXAC2 microsatellite markers and ATL1 SNP previously have been described as markers associated with Fragile X syndrome (FXS) causing gene FMR1 and CGG repeat instability. Haplotypes linked to FXS are described widely across Western European populations and Scandinavia in contrast to Eastern Europe including Baltic States. The aim of this study was to report specific haplotypes among FXS patients and patients with normal number of CGG repeat in Latvian population.

In our study were included 11 FXS patients and 122 patients with normal CGG repeat alleles. Microsatellite markers were genotyped on ABI 310 genetic analyser and results validated by direct sequencing of random alleles for each locus. ATL1 SNP amplified by allele specific PCR and analyzed on agarose gel. Gray zone alleles (35-50) were analysed for AGG interspersion pattern by direct sequencing.

In total 27 different DXS548-FRAXAC1-ATL1-FRAXAC2 haplotypes were detected - 26 in control group, 3 in FXS group. The prevalent haplotype in control group was 7-4-A-5+ (rel. frequency 0.327). The prevalent haplotype in FXS group was 2-2-G-4 (rel. frequency 0.818). Gray zone alleles with long uninterrupted CGG tract on 3` end were statistically significant associated with 2-2-G-4 haplotype. This finding let us hypothesize instability of these CGG alleles in next generations. Statistical results of our study indicate significant difference among FXS and control group. Most frequent haplotype in FXS group differs from literature data of European populations. Analysis of FXS chromosomes across Baltic States would be desirable.

P10.29
Molecular phylogenetic analysis of the Iranians and other populations

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The allele frequency of polymorphic markers has proven to be useful for survey of human migration and genetic origin. Moreover, the estimation of genetic distance between populations could improve our viewpoint about their genetic origin. In this study, we used allele frequency data of 12 polymorphic markers on 250 (500 alleles) individuals from the Iranian population to estimate genetic distance between the Iranians and other world populations. The phylogenetic trees for three different sets of allele frequency data were constructed. Our results showed that the Iranians had genetic similarity with some European populations, of which the lowest genetic distance of the Iranians with the studied populations was observed with some populations reside in Russia. Furthermore, the high genetic distance was observed between the Iranians and East Asian populations. The data suggest that the Iranians might have relatively close evolutionary history with Europeans, but historically independent from East Asian populations. This study could provide a new insight into the evolutionary history of the Iranian population.

P10.30
Genetic epidemiological study of hereditary disorders in Russian populations

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Genetic epidemiological study the prevalence's of AD, AR and X-linked disorders in some regions (7 ethnic groups) of Russia was established: Russian from seven populations, Adygean, Maris, Chuvashes, Udmurt, Bashkirs, Tatars (more than 3 millions of inhabitants). Genetic differentiation between populations of different hierarchical levels in the load of Mendelian hereditary diseases (HDs) was established. The prevalence rate of all HDs varied in the investigated populations from 1.34 to 9.78 per 1000 persons. Simultaneously with medical genetic study the population genetic study was performed. Correlation analysis between prevalence rates of AD and AR HDs and random inbreeding F_{st} as well as index endogamy was carried out. It was proposed that the genetic drift is probable one of the factor which determined genetic differentiation of populations by the prevalence of autosomal (AD and AR) HDs. Genetic diversity of HDs in the investigated populations was revealed 480 disorders (7300 affected). Basic part of load (56 % of patients) for all types of inheritance concern to the common 28 forms (15 with AD, 8 with AR and 5 with X-linked inheritance) of the HDs causing only 5,83 % of all registered disorders. However, frequencies of these 28 disorders also varied between regions. The HDs which are finding out locally high frequencies in some populations/ethnic groups were submitted. Specific common diseases and accumulation of some HDs in several populations/ethnic groups were revealed in each population. It has been demonstrated that the genes of HDs are a promising tool for characteristic ethnogenetic processes in populations.

P10.31
Gilbert syndrome molecular diagnostics and clinical characterization in Latvia

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Gilbert syndrome or benign hyperbilirubinemia is caused by changed number of (TA) repeats in UGT1A1 gene promoter - (TA)5 and (TA)6 is associated with high enzyme activity, but alleles with 7 or 8 repeats - with low activity. Gilbert syndrome is usually described as autosomal recessive disease.

Aims: to characterize Gilbert syndrome patients by biochemical and molecular data. To compare molecular genotyping methods for Gilbert syndrome.

Materials and methods. 500 DNA samples from patients that were sent with elevated bilirubin levels. 400 control individuals. DNA was analyzed by SSCP, direct sequencing and fragment analysis using ABI PRISM 300 (Lin J. P. et al 2006).

Results. Comparing genotyping methods for UGT1A1 gene the best method was found to be fragment analysis using ABI Prism 300. Between patients with changed bilirubin levels diagnose of Gilbert syndrome was approved for 90%. Main clinical complaints were icterus at stress situations, fatigue, very rare as complaints were mentioned pain. Comparing bilirubin levels there were found statistical significance between individuals with genotype (TA)6/(TA)6 and heterozygous and homozygous (TA)7 individuals. Gilbert syndrome frequency in population of Latvia is 10%. There are found alleles (TA)5 and (TA)8 that is uncommon in European descent.

Conclusion: fragment analysis is most cost and time effective method for Gilbert syndrome genotyping. Clinical picture doesn't differ from already described picture from other populations. Alleles that is found in UGT1A1 gene includes alleles that are described in Africa descent.

P10.32
Mutation analysis of the mtDNA A1555G,a3243G,and A7445G mutations in nonsyndromic sensorineural subjects in north Iran .

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Three mitochondrial DNA (mtDNA) mutations including A1555G,a3243G,and A7445G occurring in MTRNR1,MTTL1 and MTTS1 genes are considered to be the main causes of mitochondrial hearing loss in some populations. However variouse frequevcies of the mtDNA mutations have been reported from different populations. This study aims to determine the frequency of the A1555G,a3243G,and A7445G mutations in nonsyndromic sensorineural hearing loss subjects in Gilan province in north Iran .

Materials and methods: Fourty six nonsyndromic sensorineural subjects were screened for the presence of mtDNA A1555G,a3243G and A7445G mutations using PCR-RFLP procedure and subsequent direct sequencing.

Result: We found no mtDNA A1555G,A3243G,and A7445G mutations in the cohort of 46 deaf individuals studied .However PCR-RFLP of the MTTL1 gene represented a G3316A mutation due to destroying a

restriction site in the A3243G PCR fragment examined.
Conclusion: Our finding indicate that the association of mitochondrial mutation with deafness is very low in deaf subjects, in north Iran .

P10.33
FV, Prothrombin and *MTHFR* gene mutations in patients with thrombosis risk from Middle Black Sea region of Turkey

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FV (Factor V), *Prothrombin* (Factor II) and *MTHFR* (Methylene tetrahydrofolate reductase) genes mutations are listed among the causes of hereditary thrombophilia, so these genes can be used in population-based large-scale genotyping studies. We aimed to determine the frequencies of thrombophilic gene mutations in the middle Black Sea region of Turkey.
In this study we screened 2070 patients (1373 females, 697 males) sent to our laboratory from various clinics of Ondokuz Mayis University, Faculty of Medicine for genetic testing for thrombosis risk between February 2005 - November 2010. *FV* Leiden (G1691A), *Prothrombin* G20210A and *MTHFR* C677T gene mutations were analyzed using reverse hybridization Strip Assay (1818 patients) and real-time PCR (252 patients).
Embolism (32.1%), coronary artery and heart diseases (14.3%), abortion (14.1%), anemia (7.2%), infertility (5.5%), thrombosis (4.9%), malign neoplasia (4.0%) were among the reasons of genetic test request. The percentages for heterozygosity and homozygosity for *FV, Prothrombin,* and *MTHFR* gene mutations were, in order, 18.2, 7.1, 43.0, and 1.9, 0.1, 10.3, respectively.
Determining the distribution of allelic frequencies of *FV, Prothrombin* and *MTHFR* gene mutations that increase the risk of thrombosis, will contribute to understanding of the frequency of these mutations in middle Black Sea region of Turkey.
Keywords: Thrombophilic genes, mutation frequencies

P10.34
Neuromuscular Disorders in Rostov Region (Russia)

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Research of Hereditary Neuromuscular Disorders (HND) is especially important because of their prevalence among neurological disorders is high, most neuromuscular disorders have a progressive course with a lethal outcome. The population of 12 districts was examined to access prevalence of HND in Rostov Region. Total size of investigated population was 497460. Data collection and processing were done according to the protocol of Genetic Epidemiology Laboratory of Medical Centre in Moscow. The HND in Rostov Region's population compose 80% of all hereditary nervous system diseases. Their prevalence is 25,73 per 100,000. Hereditary motor and sensory neuropathy (HMSN) is the most common HND in Rostov Region. This study identified 54 cases of HMSN in 26 families. The prevalence was 10,86 per 100,000. DNA testing was performed to identify duplication of the gene *PMP22* in all patients. The mutations were found in members of 6 families. Myotonic dystrophy (MD) is the most common adult-onset muscular dystrophy. We found 21 cases with clinical manifestations of MD. The prevalence was 4,22 per 100,000. We observed tremendous variability in the phenotypic expression and severity of the disease, especially within families. This study identified 16 cases of Duchenne/Becker muscular dystrophy in 13 families. The prevalence was 6,43 per 100,000 male. DNA testing was performed to identify deletions in gene *DMD*; 3 families with Duchenn/Becker muscular dystrophy had deletions. HMSN, Duchenne/Becker muscular dystrophy, and MD are prevailing HND among Rostov Region's population, which is also true for the Russian Federation neuropathology spectrum.

P10.35
Effective Prediction of HLA Allele Genotypes Using SNP Data for a Han Chinese Population

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Genetic variation at classical human leukocyte antigen (HLA) loci has played an important role in the regulation of fundamental molecular or cellular processes and susceptibility to various diseases. Direct typing of HLA alleles can provide insights into complex associations among genomic structure, SNP variation and variation at respective alleles. However, the excessive cost has compelled researchers to adopt more economical approaches, such as SNP-based tagging and allele prediction algorithms. In this study, we modified a statistical methodology developed by Leslie et al. (2008) and applied it to predict alleles at 6 classical HLA loci (HLA-A, -B, -C, -DPB1, -DQB1 and -DRB1) by using commercially available SNP data including Illumina HumanHap550K, Affymetrix 500K SNP, Affymetrix SNP 6.0 genotypes alone and combinations of them within the MHC region for the sample of 437 unrelated Han Chinese individuals residing in Taiwan. Our results indicated that overall, a single panel of ~160 SNPs typed across the region was sufficient for predicting HLA alleles at 6 loci with up to 98% accuracy at 4-digit resolution. In conclusion, the effective prediction algorithm provides a low-cost alternative to direct typing of HLA alleles and sheds light on building a representative estimation background of HLA allele genotypes in the Han Chinese population. Moreover, the specific prediction SNP sets may aid in disease screening and medical treatment in the future.

P10.36
Huntington Disease in Hungary

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Huntington disease (HD) is a dominantly inherited neurodegenerative disorder that results from a mutation that expands the polymorphic trinucleotide (CAG) tract in the *HTT* gene. Molecular genetic analysis is available since 1997 in our Institute in Hungary. Until the end of 2010 we genotyped 319 subjects originating from 195 families. Amongst them 149 people were tested positive with a repeat length ranging from 40 to 90 CAG repeats (46.7% of all tests). 32 subjects carried an intermediate allele (10.0% of tests), with CAG repeat ranging from 26 to 36.
Epidemiological studies report a wide variation of HD prevalence. Molecular studies suggested that different prevalence rates could be due to differences in other genetic factors responsible for the disease. In this respect, we analysed the CCG length polymorphism that is adjacent to the CAG region and compared our results to those of other European studies. The CCG7 allele was overrepresented among affected chromosomes (94.6%), while we found a low percent of the CCG10 allele (5.38%); this result is in accordance with studies performed previously on other European populations. Since the origin of Hungarian population differs from other European Caucasian ethnical groups, as founders of the ancient Hungarian state were from the eastern side of the Ural Mountains, we might expect different frequencies of the intragenic polymorphisms compared to populations of Western European ancestry. The fact that we found similar frequencies suggests that the founder mutation of HD in Hungary is of Western European and not of Asian origin.

P10.37
Investigation of CAG and CCG repeats in affected patients and risk group of Huntington disease in 61 Brazilian individuals.

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Huntington Disease (HD) is a rare fatal neurodegenerative genetic disease. In this study we determined, in 61 Brazilian individuals, the sizes of the polymorphic CAG and CCG regions and correlated these sizes to age at onset of HD. We also investigated the genetic origin of *IT15* gene (4p16.3) and calculated the minimum prevalence of HD for Ervalia City, Brazil. PCR-SSP was used for amplifiying the target regions, and amplicon size was determined by capillary electrophoresis. It was found that 13 subjects had normal alleles. Out of the 48 subjects with abnormal alleles, two had intermediate number of CAG repeats (27-35) and no HD phenotype; and 46 individuals had expanded alleles. The average age of onset of HD was 41.7 years. The correlation between number of CAG repeats and age of onset of clinical manifestations was negative (r = -0.84). There was no statistically significant correlation between CCG repeats and age at onset of HD (r = 0.26). Among those cases that had expanded alleles, 11 had alleles with reduced penetrance (36-39 CAG) and 35 had alleles with complete penetrance (> 39 CAG). Alleles of 7 or 10 CCG repeats are predominant in populations, and strong linkage disequilibrium between the CCG(7) allele and HD has been shown in western HD chromosomes. CCG(7) alleles were found in 78% of 48 individuals who had abnormal alleles. Therefore, the majority the mutated *IT15* genes for HD show putative Western European ancestry. Minimum prevalence of HD for Ervalia was nine times higher than in Europe.

P10.38
Origin and possible ways of evolution of Huntington's disease alleles in Ukrainian population

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Huntington's disease (HD) is a late onset neurodegenerative disorder resulting from an expansion of a CAG-repeats of *HTT* gene on 4p16.3. The frequencies of HD are different in ethnic groups (0,6-100:1000000). The highest ones are found in countries of European origin.

To investigate the *HTT* gene expansion alleles' origin and possible ways of evolution in Ukrainian population the analysis of allelic of CGG-repeats and "del2642" polymorphisms of *HTT* gene was conducted in a group of HD-patients with CAG-expansions (40-52 CAG-repeats) and their relatives from Ukraine. CAG-expansion was found in chromosomes 4 marked by different CCG-allele variants (7, 8, 10, 12 CCG-repeats). Linkage disequilibrium between expanded CAG-alleles and the major $(CGG)_7$-allele (83%) has been displayed. We also found that the frequency of 2642-deletion of *HTT* gene is significantly higher in the group of HD-patients than in controls. Its may be due to "Western Europe founder" effect of HD-chromosomes with $(CCG)_7$-del2642 haplotypes. High frequency of $(CGG)_{10}$-allele in HD-chromosomes (12%) may be a result of migration from populations in which the HD-chromosomes are predominantly marked by this CCG-variant (Asia, ets.). Minor CCG-alleles ($(CCG)_8$ and $(CCG)_{12}$) may appear due to step-by-step mutations of major alleles. We also studied instable intermediate CAG-alleles (27 CAG-repeats of HTT gene). It has been shown that these chromosomes (5% of normal ones) have $(CCG)_7$-del2642 haplotypes (single origin). Our findings suggest that HD-mutation in Ukraine has a recurrent origin. We also found that the *de novo* HD-expansions may arise from intermediate CAG-alleles on chromosomes 4 with $(CCG)_7$-del2642 HTT-haplotypes.

P10.39
A molecular-based estimation of the prevalence of hypophosphatasia in the European population

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The prevalence of hypophosphatasia (HP), a rare metabolic disorder due to mutations in the *ALPL* gene, has never been estimated in the European population. Only one published study evaluated the incidence of severe HP at 1/100,000 in Canada 53 years ago. Moderate forms of hypophosphatasia (mHP), including HP with moderate bone features and the mildest form odontohypophosphatasia, reflect both recessive and dominant inheritance, and are therefore expected to be more frequent than severe forms. Here we estimated both the prevalences of severe and mHP in European populations. The prevalence of severe HP was estimated at 1/300,000 on the basis of the number of cases tested in our laboratory and originating from France during the period 2000-2009. We observed a significant discrepancy between North and West Europe where the prevalence was similar to France (1/340,000), and South and East Europe where the value was significantly lower (1/1,360,000). This difference may be due to several factors among which no request of mutation analysis and molecular testing in other laboratories than ours, but also to particular recurrent mutations in North and West Europe reflecting founder effects. The prevalence of mHP was then estimated by using the proportion of dominant mutations among severe alleles and by estimating the penetrance of the disease in heterozygotes for dominant mutations. According to a genetic model with 4 alleles resulting in 10 distinct genotypes, the prevalence of dominant mHP in the European population was estimated at 1/6370, pointing out that mHP is much more frequent than severe HP.

P10.40
Estimating the prevalence of autosomal recessive disorders through mutational records and consanguinity: the Homozygosity Index (HI)

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Mutational records make it possible to estimate frequencies of disease alleles (q) for autosomal recessive disorders using a novel approach based on the calculation of the **Homozygosity Index** (HI), i.e. the proportion of homozygous patients, which is complementary to the proportion of compound heterozygous patients P(CH). To test this hypothesis, we used mutational records of individuals affected with Familial Mediterranean Fever (FMF) and Phenylketonuria (PKU), born to either consanguineous or apparently unrelated parents from six Mediterranean population samples. Our method gave q estimates very similar to those of previous descriptive epidemiological studies (Table 1). By simulation studies, the minimum sample size needed for this approach is of 25 patients with either unrelated or first cousins parents. These results indicate that the **Homozygosity Index** can give a ranking order of prevalence for autosomal recessive disorders, especially in populations with high frequencies of consanguineous marriages.

Gene	Country	inbreeding coefficient (F)	Sample size	HI	q	P
MEFV	Lebanon	0.0625	34	0.5588	0.123 (0,095[1])	1:66 (**)
		Unrelated* (0.02)	107	0.3458	0.143 (0,095[1])	1:49 (**)
	Turkey	Unrelated* (0.004-0.0096)	55	0.4545	0.03-0.073 (**)	1:1082-1:188 (1:1075[2]-1:1000[3])
PAH	Israel (Arabs)	Unrelated* (0.015-0.024)	30	0.5667	0.014-0.023 (**)	1:4785-1:1838 (1:8200[4])
		0.0625	8	0.875	0.0126 (**)	1:6344 (1:8200[4])
	Israel (Jews)	Unrelated* (0.001)	87	0.2299	0.007 (**)	1:6061 (1:12500[4])

Table 1. Total allele frequencies (q) and prevalences (P) estimated by the present method or (traditional methods). (*F chosen among previously published inbreeding data; **data not available; [1]Mattit et al., 2006; [2]Ozen et al, 1998; [3]Dinc et al, 2000; [4]Berchovich et al., 2008).

P10.41
Association between K469E and R241G polymorphisms of intercellular adhesion molecule 1 and inflammatory bowel disease in Iran

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Introduction: Inflammatory bowel disease (IBD) consists of ulcerative colitis (UC) and Crohn's disease (CD), two chronic idiopathic inflammatory diseases of the gastrointestinal tract. Among various genes contributing in IBD susceptibility, Intercellular adhesion molecule (ICAM)-1 and its polymorphisms are of concern. ICAM-1 gene is located on chromosome 19p13 and plays a pivotal role in the inflammatory processes. There are two single base polymorphisms of ICAM-1 gene (G241R and K469E). In this study, we examined the association between G241R and K469E, and IBD in Iranian patients.
Material and Method: In this case-control designed study, 156 IBD patients (110 UC and 46 CD patients) and 131controls were enrolled. The study was performed in Shaheed Beheshti University during 2006 - 2009. The polymorphisms (G241R and K469E) were assessed by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP).
Results: The mutant allele of R241G was more frequent in CD patients compared to controls (33.7% vs. 22.9%; p=0.042; OR=1.71; 95%CI: 1.02-2.88) and K469E mutant allele frequency was significantly higher in CD patients compared to controls (55.4%vs. 40.5%; p=0.013; OR=0.546; 95%CI: 0.34-0.88). No difference was observed among UC patients compared to controls.
Conclusion: Our findings suggest that both mutations of ICAM1 gene may probably participate in pathogenesis of Crohn's disease in Iran

P10.42
Relationship of *iNOS* gene expression with *TNF-α* and *iNOS* gene polymorphisms among Iranian Mazandarani and Turk populations

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The identification of genetic alterations in different populations could be useful in recognizing the therapeutic aspects of drugs as well as estimating the prevalence of a variety of diseases. The promoter polymorphisms of -308G/A in *TNF-α* gene as well as -954G/C and CCTTT repeat polymorphism of *iNOS* gene were studied in this research. Due to similar conditions of *TNF-α* and *iNOS* production, the polymorphisms of these genes were studied in both Iranian Turk and Mazandarani ethnic groups. In addition, the expression of *iNOS* gene in transcriptional level has analyzed in comparison with *iNOS* polymorphism genotypes.
To identify the genetic polymorphisms, the PCR-RFLP and sequencing based method were used. The expression of *iNOS* gene was measured by real-time PCR. The results revealed that allele frequency of polymorphic -308A allele of *TNF-α* in Turk and Mazandarani ethnic groups was 15.5% and 16.3% respectively. The polymorphic -954C allele of *iNOS* gene was not observed in both ethnic groups. The most prevalent CCTTT repeat number in promoter region of *iNOS* gene in both groups was shown as 14 repeats. The transcriptional expression of *iNOS* was higher in presence of 15 repeats of CCTTT rather than 14 repeats. Moreover, it was shown that the presence of A allele at -308 position of *TNF-α* gene is associated with lower expression of *iNOS* in transcriptional level (p <0.001). In conclusion, the results of this study suggest the effect of *TNF-α* gene polymorphism on *iNOS* gene expression and this can be modified the inflammation process in the cell.

P10.43
Genetics population flows in the region comprised by the former Kingdom of Granada

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The study of genetic markers allows the characterization of the populations as well as the genetics relationships between them.
A lot of authors have studied the genetic relationships established between North African and South European people as well as the relationships with Iberian Peninsula inhabitants in order to determine the genetic legacy that remains in the present population. But, nowadays, there are not any studies which explain the genetic relationship between the population of Granada, Málaga and Almería (GMA), former Kingdom of Granada; and all those African people who inhabited in this region for more than 700 years and had an influence in its creation and establishment.
That is why, 15 STR genetic markers from 100 non-related individuals (aSTR), residents of Granada, Málaga and Almería, have been studied. Observed-allelic frequencies and common-use forensic parameters have been analyzed. Furthermore, genetic structure and its homogeneity have been studied. For a better characterization of our population, GMA population has been compared with North African, Iberian Peninsula, South European populations. Genetic distances between all the populations have been calculated. Moreover, a correspondence analysis of the allelic frequencies of the genetic markers has been executed.
By the analysis of all the results, it can be affirmed that, owing to the studied genetic markers, the GMA population maintains characteristics that show the genetic influence of North African people, even though our population resembles the rest of the Spanish population.

P10.44
Epidemiological study of Leber's Hereditary Optic Neuropathy in the Novosibirsk District, Russian Federation

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A 10-year epidemiological study of Leber's Hereditary Optic Neuropathy (LHON) in the Novosibirsk District, Russian Federation revealed the prevalence of the disease is at least 1/45,000. We have found primary LHON mutations (3460, 11778 and 14484) in only 44% of LHON pedigrees and only 30% of them are caused by 11778 pathogenic mutation. So-called LHON-like patients with the clinical characteristic of LHON disease (Abu-Amero and Bosley 2006) compose 56% of our sample. Phylogenetic analysis of the entire mtDNA sequences of all patients in this study showed that certain novel and haplogroup-specific mtDNA mutations meet the criteria of the pathologic mutations. Our data imply that genetic modifiers, such as haplogroup-specific mutations in mtDNA and various mutations in nDNA-encoded mitochondrial genes, may promote disease development in Russian LHON patients.

P10.45
Interaction within LPA gene between two multiallelic polymorphisms (5'PNRP and KIV2RP) influences Lp(a) levels and is revealed as a risk factor for Myocardial Infarction in a Spanish family-based sample.

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LPA gene codes for apolipoprotein(a) (apo(a)), which constitutes part of lipoprotein(a) (Lp(a)) and influences Lp(a) bloodstream levels, important risk factor for early atherosclerosis and myocardial infarction. LPA gene chromosomal region (6q26-q27) has recently emerged as one of the top 12 loci associated with Coronary Artery Disease/Myocardial Infarction (CAD/MI) in recent Genome-Wide Association Studies (GWAS). In this work we test the influence on Lp(a) levels and the association with MI of two repeat polymorphism, 5' Pentanucleotid (TTTTA) repeat polymorphism (LPA 5'PNRP) and Kringle IV-type2 domain repeat polymorphism (LPA KIV2RP), in 101 family trios from Spain with younger than 55 MI affected descendent (N=302 individuals). The polymorphism were not in LD. Significative correlation showed an inverse relationship between Lp(a) levels and mean repeat number of 5'PNRP and KIV2RP in the whole sample, but only for 5'PNRP in the patients sample. The analyses of variance showed a significative interaction between mean repeats of 5'PNRP

and KIV2RP for Lp(a) levels in the patient sample, explaining the 63,4% of the observed variance of Lp(a) levels. In order to test for Transmition Disequilibrium (TDT) the alleles were grouped into categories. No transmition deviation was observed for the single alleles. But, it was observed overtransmition for the most frequent haplotype (0.197) (p=0.007), which grouped the shorter alleles of both polymorphisms (5'PNRP*8 repeats and KIV2RP*<23 repeats). This results revealed an interaction effect between the shorter repeats of 5'PNRP and KIV2RP on the Lp(a) levels and, in consequence, on the risk of early atherosclerosis and myocardial infarction.

P10.46
Human lymphatic filariaisis: Genetic Polymorphism of Endothelin-1 and TNF receptor II correlate with development of chronic disease

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Background: Hydrocele and elephantiasis are two clinically very diverse and often mutually exclusive chronic manifestations of human bancroftian filariaisis. Plasma levels of Endothelin-1(ET-1), a major angiogenic factor and TNF receptors (TNFRs) that regulate host inflammation have been associated with development of chronic filariasis although their genetic basis are not known.
Methods: We studied polymorphisms of ET-1(Ala288Ser) and TNFRII (Met196Arg) genes by means of polymerase chain reaction confronting two pairs primers method and restriction fragment length polymorphism respectively. Plasma ET-1 levels was measured by Enzyme-linked immunosorbent assay.
Results: Met196Arg genotype frequency of TNFRII polymorphism was significantly more in hydrocele cases in comparison to elephantiasis patients (OR: 4.34 and 95% CI: 2.04 to 9.20). Conversely, significantly high prevalence of Ala288Ser mutation of Endothelin-1 was observed in elephantiasis patients as compared to hydrocele cases (OR: 2.15 and 95% CI: 1.13 to 4.10). Decreased plasma ET-1 levels correlated significantly with Ala288Ser mutation in the study population. A combined analysis indicated a 23 fold for higher risk for developing elephantiasis in subjects with TNFRII (Met196Met) and ET-1(Ser288Ser).
Conclusions: ET-1 (Ala288Ser) and TNFRII (Met196Arg) polymorphisms are associated with development of one or the other forms of chronic disease in bancroftian filariasis.

P10.47
Mannan-binding lectin serine peptidase 2 gene (MASP2) polymorphisms in rheumatoid arthritis patients from Brazil.

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Aims. Rheumatoid arthritis (RA) is an autoimmune disease (AID) which affects at least 1% of the world's population. The immunopathogenesis of RA is not entirely clarified but the importance of complement system in its development is unquestionable, although still poorly understood. The present study aimed to evaluate the prevalence of MASP2 gene polymorphisms in patients with RA from Brazil.
Methods. Nine MASP2 polymorphisms distributed from the promoter to the last exon were haplotyped through multiplex PCR amplification with sequence-specific primers. A total of 156 RA patients (24 male, 132 female, mean age 51 [24-65] years) and 125 healthy individuals (18 male, 107 female, mean age 43 [24-69] years) were investigated. The study was approved by the local ethics committee.
Results. We identified 10 different MASP2 haplotypes with genotype distributions in Hardy and Weinberg equilibrium. There was a trend for higher frequency of MASP2 haplotypes with the p.D120G and p.P126L polymorphisms, causing low MASP-2 levels in the RA patients (Fisher exact test = 0.06), but no difference was found in the frequencies of high-, low and intermediate MASP-2 producers between patients and controls.
Conclusions. In the present analysis, we did not find an association of MASP2 polymorphisms and haplotypes with RA disease.

P10.48
The utility of commercial STRs beyond forensic purposes: Population relationships in North Africa

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The variability of 15 commercial STRs used for forensic purposes has been employed in this work to analyze the relationships of different Arabic and Berber-speaking North African ethnic groups. A total of 165 individuals from the Doukkala and Khenifra Moroccan regions have been genotyped using the AmpFISTR identifiler kit (Applied Biosystems, Foster City, CA, USA). Data from these two samples completed a previous genetic database of five North African groups genotyped for a variety of autosomal polymorphisms (STRs, Alu, and Alu-STR combinations). The joint use of markers with different mutation rates could be very useful to detect ancient relationships and/or more recent demographic events such as gene flow. Our results underlined the genetic distinctiveness of North African samples (Morocco and Algeria) from North (Spain) and South East Mediterranean groups (Egypt). Differences in genetic heterogeneity estimates strengthened the necessity of taking into account information from genetic markers with different mutation rates to explain properly human population events. In particular, in those populations that share close geographic and historical relationships.

P10.49
Prevalence of rs41274239 polymorphism in the miRNA gene *miR96* in different ethnic groups in Siberia and in patients with common diseases

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MicroRNAs are short noncoding RNAs which are involved in posttranscriptional regulation of gene expression. The primary transcript of miR96 (7q32.2) generates miR96 and antisense product miR96* (miRBase:MI0000098). SNP rs41274239 (+36A/G) is located in the loop-like structure of pre-miR96; minor allele frequency is 2.8% (NCBI database). We have studied prevalence of this polymorphism in Siberian populations (Russians, Buryats, Yakuts. Tuvinians, 352 individuals in sum). The minor allele frequency in Russians was estimated as 1.05%, with only heterozygous genotypes; in other populations (all of mongoloid origin) the polymorphism was not found. We have studied also this SNP frequency in Russians with different diseases: ischaemic heart disease, hepatitis C, tuberculosis, asthma (126, 173, 207, 144 DNAs, respectively). The polymorphism was very rare in all groups, being registered only in heterozygous state with frequency from 0.24% in tuberculosis to 0.69% in asthma. In the pooled sample of patients, heterozygosity was 0.0094 +/- 0.0035; minor allele G frequency 0.47%. The present study is the first representative work on this SNP. The results show that the G allele of rs41274239is rare in Caucasians and was not registered in Mongoloid Siberian populations.

P10.50
Possible functional differences of the two mitochondrial DNA polymerase gamma (POLG) gene haplogroups

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About 150 mutations in the human mitochondrial DNA polymerase gamma (POLG) have been identified in patients with mitochondrial diseases such as Alpers syndrome, progressive external ophthalmoplegia, and ataxia-neuropathy syndromes. However little is known about the haplotypic structure of this gene. Here we have sequenced *POLG1* gene 5'-promotor region and analyzed distribution of this gene haplotypes in two North Eurasian populations - in Russians (n=63) and Buryats (n=90). Only one polymorphic locus, namely rs2856268, was revealed in the 5'-promoter region studied. Comparison with our previous haplotypic data has shown that the alleles

rs2856268-G and -A are strongly associated with haplogroups A and B of the *POLG1* gene. Interestingly that association of the rs2856268 variants with diabetic polyneuropathy (DPN) in type 1 diabetes mellitus has been recently reported in Russians (Spitsina et al. 2009). It was found that the carriers of G-allele and GG-genotype had higher risk of DPN development than the carriers of A-allele and AA-genotype. One may suggest that the polymorphism in the 5'-promotor region can differentially modulate expression of the whole *POLG1* gene, pointing to a higher possibility for G-allele to determine a lower level both of the *POLG1* gene expression and the mtDNA repair. In addition, taking into account that haplogroups A and B could evolve separately for a long time (about 1.5 millions of years), it is important that possibly more accurate form of enzyme is associated with younger and more frequent in humans haplogroup B. This work was supported by the grant from FEB RAS (09-3-A-06-221).

P10.51
HaploGrep - automatic classification of mitochondrial DNA haplogroups

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Background: Human mitochondrial DNA (mtDNA) is routinely analyzed in various disciplines, such as medical genetics, population genetics and forensic genetics. For studying mechanisms of human evolution, discovering genes involved in complex diseases and quality management, the determination of the mtDNA haplogroup is a critical requirement. However, despite the availability of Phylotree, a regularly updated classification tree of global mtDNA variation, the process of haplogroup classification is still time-consuming and error-prone, as researchers have to manually compare the polymorphisms found in a population sample to those summarized in Phylotree, polymorphism by polymorphism, sample by sample.

Results: To eliminate these shortcomings we designed HaploGrep, a fast and reliable algorithm to determine the haplogroup affiliation of thousands of mtDNA profiles genotyped for the entire mtDNA or any part of it. HaploGrep is implemented as a freely available web application, uses open source technologies and scales well in time and space. For every input sample the top 10 results and the phylogenetic position of the respective haplogroups are displayed, thus providing a detailed explanation of how and why a haplogroup was ranked best. Furthermore, numerous export possibilities are given and all results are visualized in a tree structure, showing the current position in Phylotree with hints for further refinement of the actual haplogroup status and potential errors in the classification process.

Conclusions: HaploGrep uses the latest version of Phylotree and offers an all-in-one solution for quality assessment of mtDNA profiles in clinical genetics, population genetics and forensics. HaploGrep can be accessed freely at http://haplogrep.uibk.ac.at

P10.52
Autosomal and uniparental genetic diversity of the populations of Sakha (Yakutia): Implications for the peopling of Northeast Eurasia

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Sakha Autonomous Republic occupies a quarter of Siberian total land area in its northeastern part, is an important region for understanding the colonization of the Northern Eurasia by anatomically modern humans. To characterize the genetic variation in Sakha both the haploid mitochondrial DNA (mtDNA) and Y chromosomal as well as diploid autosomal loci (650 000 SNPs) of genome were analyzed in five native populations of Sakha (Yakuts, Evenks, Evens, Dolgans and Yukaghirs).

While striking prevalence of Y chromosome haplogroup N1c in gene pool differentiates Yakuts from other populations, the mtDNA and autosomal analyses demonstrate genetic similarity of all native populations of Sakha, in particular Yakuts and Evenks. The results also demonstrate closest genetic proximity of the populations of Sakha with southern Siberians. Both mtDNA and autosomal analyses reveal deep genetic discontinuity between Siberian and Beringian populations. MtDNA haplogroups A2 and G1b, prevalent in Beringian populations, are either minor or even absent in Sakha, where haplogroups C and D dominate. Autosomal analysis also differentiates Beringian populations from those of Sakha. Our results support the scenario that the territory of Sakha was colonized from the regions west and eastward of Lake Baikal with only minor gene flow from Lower Amur/Southern Okhotsk region and/or Kamchatka.

P10.53
Genetic epidemiology of DFNB1-associated hearing loss in Russia

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The major objective of this study was to determine contribution of DFNB1 locus allelic variants to development of non-syndromic hereditary hearing loss (NSHL) among Russian patients of different populations. 228 NSHL patients were analyzed. 35delG mutation was responsible for development of disease in few cases (Chuvash Republic - 28,6%; Republic of Bashkortostan - 0,0%; Republic of Udmurtia - 50,0%; Kirov Region - 26,8%; Rostov Region - 19,2%).

We determined the effect of other mutations in DFNB1 in NSHL progression in different groups of Russians. We analyzed coding exon of Cx26. As the result, the number of patients with identified alleles achieved 35.8% in Kirov Region and 30,8% in Rostov Region. Second mutation has been identified in almost all patients heterozygous for 35delG. However, spectrum of mutations differed in investigated areas. We analyzed noncoding exon of Cx26, but no changes were observed.

We have shown the differences in allele frequencies involved in formation of DFNB1-associated NSHL in different Russian patients. Furthermore, sequencing of coding exon of Cx26 increased the proportion of patients with a confirmed diagnosis beside the analysis of 35delG mutation only. Involvement of Cx30 wasn't confirmed in this type of NSHL development.

Based on our investigation, we could postulate the feasibility of testing of the coding exon of Cx26 in DFNB1-patients of Russian nationality during genetic diagnosis. It's required to search for additional mutations in DFNB1 which are not detected by sequencing of Cx26 and Cx30, as well as in other genes involved in genetic interactions with Cx26.

P10.54
Prevalence monogenetic syndromes of multiple congenital malformations in population of Rostov region (Russia)

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A total medical genetically study of the population of the Rostov region was carried out (12 districts - 497,460 total investigated persons). Monogenic syndromes of multiple congenital malformations (MMCM) was examined. The overall prevalence of MMCM was 1:2292 persons. Among the syndromes MMCM with autosomal dominant (AD) inheritance type was identified 29 diseases in 129 patients, with autosomal recessive (AR) inheritance type was identified 19 diseases in 32 patients, with X-linked type of inheritance - 6 of diseases in 15 patients. Sporadic syndromes MMCM was identified in 41 patients (6 diseases). Among frequent syndromes MMCM with AD type of inheritance were revealed Noonan syndrome (prevalence of 1:18424), Aarskoga syndrome (1:41455), Opitz BBBG syndrome (1:55273), Klippel-Feil syndrome (1:55273), Greig cephalopolysyndactyly syndrome (1:71066), Waardenburg syndrome, type I (1:82910), hemifacial microsomia (1:82910), EEC syndrome (1:82910), LEOPARD syndrome (1:82910). Some syndromes had met with a lower prevalence (1: 100001-1:200000): camptodactyly with muscular hypoplasia, skeletal dysplasia, and abnormal palmar creases, Pfeiffer

syndrome, Saethre-Chotzen syndrome, Beckwith-Wiedemann syndrome, velocardiofacial syndrome, Williams syndrome, Prader-Willi syndrome, frontonasal dysplasia. Among the most frequent syndromes MMCM with AR inheritance type were identified Dubowitz syndrome (1:99492), Coffin-Siris syndrome (1:165820), Seckel syndrome (1:165820). Among the most frequent syndromes MMCM with X-linked type of inheritance were identified Lujan-Fryns syndrome (1:82910), Coffin-Lowry syndrome (1:165820). Among the frequent sporadic form of syndromes MMCM were identified constricting bands, congenital (1:17154), Pallister-Killian syndrome (1:165820), VATER-association (1:165820). These results agree with data previously surveyed populations of Russia.

P10.55

Association of *OS*, *ESR1*, *COL1A*1 and *CALCR* gene polimorphisms with osteoporosis in postmenopausal women

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Osteoporosis is a common multifactorial disease characterized with reduced bone mass. The purpose of this study was to examine the relationship between osteoporosis and variants of osteocalcin (*OS*) -298 C>T, estrogen receptor 1 (*ESR1*) 397T>C, collagen type 1 alpha 1 (*Col1A1*) 2046G>T and calcitonin receptor (*CALCR)* 1340T>C. Blood samples collected from 158 postmenopausal women with osteoporosis and 108 postmenopausal healthy female controls were analyzed by PCR-RFLP method.

ESR1 CC genotype compared with TT + TC genotypes were found to increase two fold the risk of osteoporosis. [p= 0.039 OR=2.156 %95CI (1.083-4.293)]. *CALCR* CC genotype compared with TT+TC genotypes were found to be protective effect against osteoporosis [p=0.045 OR=0.471 %95CI (0.237-0.9372)]. There was no statistically significant difference in the genotype and allele frequencies of patients and controls for *OS* -298 C>T (p= 0.293, p= 0.437, respectively) and *Col1A1* 2046 G>T polymorphisms (p= 0.283, p= 0.491, respectively). In the combined genotype analysis, *ESR1/CALCR* TCCC combined genotype was found to be protective effect against osteoporosis [p=0.0125 OR=0.323 %95 CI (0.1383-0.755)]. However, *OS/Col1A1* CCTT and *ESR1/CALCR* CCTT combined genotypes were risk factors for osteoporosis (p=0.027, p=0.009).

According to the results of our study, *ESR1* CC genotype and *OS/Col1A1* CCTT, *ESR1/CALCR* CCTT combined genotypes cause a predisposition to osteoporosis. However, CC genotype of *CALCR* gene and *ESR1/CALCR* TCCC combined genotype have a protective effect against osteoporosis. This is the first study in Middle Black Sea region, by enlarging the study population we hope that more explanatory and definitive results can be obtained.

P10.56

Different patterns of TLR2 polymorphisms in populations of various ethnic and geographical origins

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BACKGROUND: Innate immunity is triggered upon invasion of the host by microorganisms through pathogen recognition by pattern recognition receptors (PRRs). The best studied class of PRRs, Toll-like receptors (TLRs) recognize specific pathogen-associated molecular patterns (PAMPs) from various microorganisms. Genetic variation in TLRs may influence susceptibility to infections. We studied genetic variation of TLR2 in various populations and whether this variation may be due to genetic drift or natural selection.

MATHERIAL AND METHODS: We have recruited a total of 776 individuals from different populations classified according to geographic and ethnic criteria into 7 groups: Romanians, Vlax-Roma, Dutch (European populations), Han-Chinese (East-Asia), Dogon, Fulani (Africa), and Trio-Indians (America). We genotyped three non-synonymous TLR2 polymorphisms: 1892C>A, 2029C>T and 2258G>A. To avoid false-possitive results for 2029C>T we have also sequenced the TLR2 non-coding exon 3 duplication.

RESULTS AND CONCLUSIONS: The 2029C>T seems to be absent among both European and non-European populations, with the exception of the Vlax-Roma, suggesting that this polymorphisms most likely aroused in Indo-Aryan people after migration into South-Asia. Due to very low frequency of the minor allele (1.3%), it was difficult to identify enough individuals bearing this mutation, in order to perform experiments on its functional effects. 1892C>A was found exclusively in European populations, but not in Asian, African or American volunteers, probably occurred in Proto-Indo-Europeans. Interestingly, 2258G>A was present only in Europeans, but not in Vlax-Roma. The differential pattern of the various TLR2 polymorphisms in various populations may explain some of the differences in susceptibility to infections between these populations.

P10.57

Phylogeographic Analysis of Mitochondrial DNA in the Arabian Peninsula

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Phylogeographic analyses of mitochondrial DNA (mtDNA) provide insights into modern human evolution. In recent years, worldwide studies of contemporary mtDNAs have indicated that modern humans left Africa ~60,000-70,000 years ago along the "southern coastal route", across the Red Sea and via the Arabian Peninsula. Yet no obvious signs of the passage though Arabia have been found in genetics and archaeology fields. The aims of this work are to seek for possible mtDNA relicts of the initial dispersal from Africa in Arabia and to investigate the origins of lineages that arrived later. We are doing this by sequencing the complete mtDNA molecule (~16,568 bp) from unclassified lineages (referred to as the paraphyletic clusters L3*, N* and R*) and poorly studied haplogroups within the Eurasian macrohaplogroup N, which is predominant in Arabian populations today (86% in Saudi Arabia, 66% in Yemen and 79% in Dubai), in 90 samples from Dubai, Yemen, North/East Africa, the Near East and Europe. Our results will allow to test hypotheses about the settlement of the Arabian Peninsula.

P10.58

Prevalence of hereditary pathology among the childrenâ€™s population of Bashkortostan Republic of Russia

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Results of prevalence of a monogenic hereditary pathology (MHP) among the children's population of Republic Bashkortostan (eight Districts) was submitted. The total size of investigated population was made 250110 people (64935 children). All Districts were examined by standard protocol of medical genetic research elaborated in laboratory of genetic epidemiology, Research Centre for Medical Genetics. About 2800 MHP and syndromes of OMIM could be identified by this research. Clinical investigations were performed by neurologists, ophthalmologists, orthopedic, otolaryngologies, dermatologists, pediatricians and clinical geneticists, focused on diagnostic of MHP. The load of children's population by all types MHP - autosomal dominant (AD), autosomal recessive (AR), and X-linked, separately for urban and rural populations was calculated. Total load of AD and AR disorders was 3.87Ã‘Â±0.24 and 2.51Ã‘Â±0.20 per 1000 children, X-linked disorders 0.92Ã‘Â±1.17 per 1000 boys. Our analysis showed that the total load of MHP in children is very high, and close to 1.4%. The spectrum of MHP detected in the eight Districts comprises 113 nosologycal forms. Spectrum of MHP was analyzed according to age of manifestation of disease, life expectancy of patients and fitness. The

total fitness of patients with AD pathology is close to unity (0.87), in families with AR pathology was 0.04, with X-linked disease was 0.16. Patients with low-fitness genotypes are rarely found among patients older than 30 years, and practically do not occur among patients older than 40 years. In this case, the reduction in fitness caused, as a low survival rate and almost zero fecundity.

P10.59
The genome sequence and assembly of world's oldest woman: a longevity reference genome

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Human genetic diversity has fundamental implications for understanding the genetic basis of diseases. Much effort is currently spent on identifying the genetic variants that are associated with longevity. However, an anonymous reference such as Hg19 can introduce a reference bias in longevity studies; a de novo assembly of a unique genome coding for long healthy life represents a more accurate biological reference genome for such projects.

We had the opportunity to study the genome of a woman who, at the time of her death, was the oldest human being alive in the world. She lived until the age of 115 years without experiencing any problems associated with vascular disease or dementia. At the age of 112-113, she was subjected to neuropsychological examinations, which revealed that her general performance was above average for healthy adults between 60-75 years. Post-mortal examination showed almost no signs of atherosclerotic plaque formation.

We sequenced and assembled the genome of this individual thus generating an independently assembled reference genome. For this, we employed short and long insert paired end reads on the SOLiD™ System. Initial analysis of this genome is focused on identifying the presence or absence of DNA variants associated with atherosclerosis as this is the main cause of death among older people, and the associated vascular dementia is a leading cause of dementia. Ultimately, we will use this genome as an independent reference for larger longevity studies such as the Scripps Wellderly study.

P10.60
MTHFR C667T polymorphism, folate level and hypertension in Croatian elderlies

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The Methylenetetrahydrofolate reductase (MTHFR) enzyme is involved in reducing homocysteine level which, when elevated, has been found to predict cardiovascular events. Adequate blood folate status may decrease the circulating level of homocysteine and lower the incidence of cardiovascular diseases (CVD). We studied MTHFR gene variant C667T, its association with hypertension (HT) and CVD endpoints in Croatian elderly population (85-101 yrs; mean=88.23 yrs; N=303; 65.3% hypertensives). Compared to the previously reported data, the TT genotype frequency was twice as high in senescents as in Croatian general population (12.5% vs. 6%, p=0.023). The results of multiple logistic analysis in elderlies showed significance of younger age (<90 yrs) and TT genotype (OR 2.175, p=0.039) in predicting HT. T allele carriers had increased incidence of stroke in comparison to non-carriers (p=0.027). Further, we evaluated associations between MTHFR genotype, serum folate and HT. Regardless of association of low folate (<7 pmol/l) and TT genotype (p=0.002), we found no association of folate and HT. According to our results, adequate folate levels in blood serum have no protective effect on incidence of HT. Therefore, we suggest that further research of folates - CVD endpoints relation should be directed towards broader spectrum of genes involved in folate and homocysteine metabolism rather than focusing on MTHFR polymorphisms as the main factor. Also, our results indicate that T allele may have some selective advantage that contributes to longevity.

P10.61
Role of some apoptosis genes polymorphisms in aging and longevity

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Aim of study was to estimate alleles and genotypes frequencies dynamic of SIRT1 (-1138C>T), NFKB1 (28081564T>A), TP53 (P72R), Casp8 (-652(6)I/D), BAX (919 A>G), BCL2 (140016C>T) genes with age in Tatars (Bashkortostan, Russia).

Total group was composed of 1602 unrelated persons from 16 to 109 years. Gene polymorphism was analyzed by PCR-RFLP. Search of genetic markers associations with age was performed using logistic regression analysis (SPSS18.0). Increase or decrease of certain genotypes with age in periods, defined by applying ROC-analyze, was estimated in odds ratio index - OR (> 1 or <1 respectively).

In male there were increase of genotype frequencies of NFKB1*T/*T in age until 72 years (p=0.028, OR=1.014), TP53*P/*P in 16-80 years (p=0.029, OR=1.012), BAX*A/*A from 17 years (p=0.006, OR=1.01); decrease of genotype frequencies of SIRT1*C/*C in age until 58 years (p=0.026, OR=0.976), NFKB1*T/*T in age until 72 years (p=0.038, OR=0.985), Casp8*I/*I in 15-76 years (p=0.045, OR=0.991), BAX*G/*G from 26 years (p=0.026, OR=0.983), BCL2*T/*T in age until 80 years (p=0.002, OR=0.989). In female SIRT1*C/*C (until 90 years, p=0.002, OR=1.023), BAX*A/*A (from 73 years, p=0.022, OR=1.033), BCL2*T/*T (since 50 year, p=0.002, OR=1.023) genotype frequencies were rises; SIRT1*C/*T genotype frequency until 90 years (p=0.027, OR=0.987) and BCL2*C/*C genotype frequency since 65 years (p=0.009, OR=0.971) were reduces.

Thus, TP53(P72R) and SIRT1(-1138C>T) gene polymorphisms are important for achieve of senile age in men and women respectively; BAX(919A>G) polymorphic marker (in both men and women) and BCL2(140016C>T) gene polymorphism (among women) may be associated with longevity.

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P10.62
Genotype and haplotype analysis of TP53 and health related traits in Croatian senescent population

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The TP53 gene has been suggested to be associated with various diseases and with longevity due to its important functions in preserving the genome integrity. In this study we investigated the association of two polymorphisms of TP53 gene - Arg72Pro and PIN3 (+16bp) - with health related traits in 324 persons of very old age (85-101 yrs). The results showed that the health status in elderly is sharply discriminated by PIN3 (+16bp) genotypes: carriers of A1A2 or A2A2 genotype more frequently have cardiovascular diseases (CVD), osteoporosis, risk for undernutrition (weight loss, diarrhea), and they also recently suffered from stress or acute illness. In contrast, for the carriers of A1A1 genotype none disadvantageous condition could be detected. The Arg72Pro polymorphism differentiated the study participants as follows: ArgArg carriers showed more frequently CVD while ArgPro and ProPro carriers had lower body mass index and they more frequently suffered from stress or acute illness. Further analysis showed that the most frequent haplotype ArgArg_A1A1 (48.9% of all haplotypes), although very advantageous for many traits is characterized by increased fasting glucose levels or/and diabetes. The remaining two A1A1 haplotypes (ProPro_A1A1 and ArgPro_A1A1) have lower frequency of CVD and CVD risk factors as well as of osteoporosis; the only disadvantageous characteristic being an increased risk for undernutrition. The present preliminary study exposes A1A1 genotype as the most advantageous TP53 genotype while A2 allele (A1A2 and A2A2 genotypes) bears substantial risk for various health related traits and it is so irrespective of codon72 status.

P10.63
Peroxiredoxin-5 gene polymorphism and human longevity in Russian population

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Peroxiredoxin-5 (PRDX5) is a mitochondrial antioxidant enzyme that neutralizes reactive oxygen species (ROS). According to the free radical theory of aging, an accumulation of oxidative damages as a result of ROS attack is probably a reason of aging and age-related pathology. *PRDX5* gene polymorphism may cause a different activity of peroxiredoxin-5 and thus may play a role in human longevity.

The purpose of our study was to reveal associations of *PRDX5* gene polymorphism with human lifespan.

We analysed 2 SNPs in *PRDX5* gene (-540A>C, rs 28364831 and 1955C>T, rs 627425) in two groups of unrelated persons from St-Petersburg: young (4-17 years old, n=123) and elderly (80-106 years old, n=173), including long-livers (90-106 years old, n=85). Genotyping was carried out by PCR-RFLP-analysis. Linkage disequilibrium and haplotype frequencies were estimated by EH program. Allele and genotype frequencies were compared by using Chi-square test, Fisher's exact test.

We have revealed linkage disequilibrium between SNPs (D = 0,12) and only 3 alleles (haplotypes). Allele -540/1955 is absent in our population. No statistically significant differences in allele frequencies between groups were found. The genotype distributions are consistent with Hardy-Weinberg equilibrium in both groups. We found the -540/1955 genotype was significantly less frequent in long-livers compared with young group (11% vs. 24%; =0,012; OR=0,37; 95%CI: 0,16 - 0,82), with group aged 80-89 years (11% vs. 27%; =0,006; OR=0,32; 95%CI: 0,14 - 0,73) and with combined group aged <90 years (11% vs. 26%; =0,004; OR=0,34; 95%CI: 0,16 - 0,73).

P10.64
Molecular, audiological and population features of autosomal recessive deafness 1A (DFNB1A) in Yakut population isolate from Eastern Siberia

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Hereditary forms of hearing impairment (HI) causing by *GJB2* (Cx26) mutations are the frequent sensory disorders registered among newborns in various human populations. In this study we present data on the molecular, audiological and population features of autosomal recessive deafness 1A (DFNB1A) associated with the donor splicing site q.-3179 (IVS1+1G>A) mutation of *GJB2* gene in Yakut population isolate of the Sakha Republic (Yakutia) located in Eastern Siberia (Russian Federation). The Yakut population exhibits high frequency of some Mendelian disorders which are rare in other populations worldwide. Mutational analysis of *GJB2* gene in 86 unrelated Yakut patients with congenital HI without other clinical features has been performed. In this study we registered a large cohort of Yakut patients homozygous for the q.-3179 (IVS1+1G>A) mutation (70 unrelated deaf subjects in total). Detailed audiological analysis of 40 deaf subjects with genotype q.-3179 (IVS1+1G>A)/q.-3179 (IVS1+1G>A) revealed significant association of this genotype with mostly symmetrical bilateral severe to profound HI (85% severe to profound HI versus 15% mild to moderate HI, p<0.05). The extremely high carrier frequency of the q.-3179 (IVS1+1G>A) mutation (11.7%) from six investigated populations has been found in Yakut population. Reconstruction of 140 haplotypes with q.-3179 (IVS1+1G>A) mutation demonstrates the common origin of all mutant chromosomes found in Yakuts. The age of mutation was estimated to be approximately 800 years. These findings characterize Eastern Siberia as the region with the most extensive accumulation of the q.-3179 (IVS1+1G>A) mutation in the world as a result of founder effect.

P10.65
Reference population database for forensic DNA typing

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The probability of a random match between two individuals derived from a large heterogeneous population database is likely to have potentially serious errors. It is hypothesized that the genetic variation between larger populations is not different from that between smaller populations and the match probability estimates are not affected by the population substructure. In order to test the first hypothesis, allele frequency data for three STR loci (TPOX, TH01 and CSF1PO) taken from 37 other studies, were analyzed. A hierarchy of the populations (Races→Caucasoids→Caucasoid Asians→Caucasoid Indians) was constructed and statistically analyzed. (A) Significant differences (p<0.05) in the allelic frequency distribution were in order of Races>Caucasoids> Caucasoid Asians = Caucasoid Indians. (B) Significant difference (p<0.05) in average number of alleles (n_a) was observed between Races and between Caucasoids. However significant correlation was observed between sample size and the number of alleles. (C) Ratio of allelic frequencies of > two fold was in order of Races>Caucasoids> Caucasoid Asians > Caucasoid Indians. Ratio of >25 folds were observed only between Races. (D) A frequency differential of > 20% was observed only between Races and between Caucasoid Asians. Second hypothesis was tested through STR genotype data across five Pakistani subpopulations. The match probability estimates of the pooled data differ significantly (p≤0.05) from that of the subpopulations. The combined match probability also shows as much as seven folds difference from the subpopulations. The analyses showed that a reference database from a homogeneous smaller population is more likely to provide reliable estimates of match probability.

P10.66
Vlax Roma in Croatia - signs of endogamy in maternal gene pool

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Vlax Roma are a branch of Romani people who were enslaved in the historic Romanian states Wallachia and Moldavia between 14th and 19th centuries. After Roma slavery was abolished, larger Vlax Roma groups immigrated to Croatia. Since Roma population is made of numerous socio-cultural endogamous groups, we investigated the extent of fragmentation of Vlax Roma in Croatia by analysing hyper variable segment I (HVS-I) and typing relevant RFLP sites in the mitochondrial DNA of 390 Bayash from two Croatian regions: Baranja (235) and Medjimurje (155). Diversity indexes point to higher level of variation among Vlax Roma settled in Eastern (Baranja) than in Northwestern Croatia (Medjimurje). The analysis of mismatch distribution clearly points to differences in demographic changes of these two subpopulations. Those results are in concordance with field research data on residence patterns indicating higher endogamy in Northwestern than Eastern Croatia. Interestingly, collected socio-cultural data point to absence of marriage unions between Vlax Roma from Eastern and North-western Croatia, indicating that shared mtDNA lineages are a result of common gene pole that predates their separation in Croatia. Despite similar origins and shared demographic history of two Croatian Vlax Roma populations, founder effect followed by strict endogamy shaped their evident population differentiation.

P10.67
A genome-wide analysis of Sardinian population structure

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Sardinia is particular attractive for human genetic studies, being one of the larger isolated populations and thus suitable for large-scale

studies. Several attempts have been made to explore its genetic structure, but they either analyzed a large set of markers in very few samples or thousands of individuals at specific loci. Here we genotyped 2,615 individuals with the Affymetrix 6.0 array. Samples were recruited from the north, south and central east areas of the Island, and initially considered as 3 distinct populations. Genotype calling was performed with Birdseed-v2, considering all samples as a unique cluster to avoid batch effects. Subsequently, we applied standard filters for samples and SNP quality, and used IBD sharing to detect, and discard, hidden relatives. Using principal component analysis, we identified outliers and reassigned each individual accordingly. An analysis of molecular variance indicated that only 0.21% of the variability could be attributable to inter-population variation (Fst=0.002), confirming a lack of large-scale substructure. We thus considered the Sardinians as a unique sample. Compared to HapMap3 populations, as expected, higher similarity was observed with Tuscany and CEPH samples (Fst=0.005 and 0.010, respectively). A genome-wide search for SNPs highly differentiated between Sardinians and these European populations confirmed the specialness of HLA and LCT regions, and also showed elevated Fst values (>0.27) at the CR1 gene, known to be related to malaria severity. We are now integrating sequencing data of many individuals to provide a more comprehensive analysis of variants in addition to the common SNPs in current genotyping platforms.

P10.68
The theoretical value of kin in aging theory: a modification of Hamilton's equations of declining selection

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The evolutionary theories of aging proposed by Hamilton, Williams and Medawar during the last century remain our dominant explanation for the phenomenon of senescence. Hamilton, Williams and Medawar pointed out numerous shortcomings of these theories in their original papers and subsequent comments, yet since their publication little has been attempted to directly address these issues.
The criticisms levelled at these theories include an inability to explain prolonged post-reproductive survival, the evolution of menopause and early infant mortality, the existence of 'negative' and 'negligible' senescence, positive selection for cellular suicide, and an apparent tautology in defining the likelihood of death. All of these valid criticisms can be addressed using a simple modification of Hamilton's equations. The Eueler-Lotka equation and the Malthusian parameter form the definitional basis of fitness for Williams and Hamilton. Yet these equations carry an implicit and incorrect assumption: that the production of offspring is the measure of fitness for an individual. This assumption creates a situation where fitness must always be a positive, declining vector.
By the simple introduction of an indirect fitness term, and the removal of the dx variable, the traditional equations of Hamilton become more accurate measures of fitness and can account for all of the above phenomena.

P10.69**
Investigating genetic modulation of sudden cardiac death by variants discovered in genome wide association studies for ECG indices of conduction and repolarization

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Background: Sudden cardiac death is a leading mode of death in adults in the Western world and is largely caused by ventricular fibrillation(VF) during acute myocardial ischemia-infarction(MI). Although a genetic component in risk of VF in MI is recognized, underlying genetic factors remain largely unknown. ECG parameters predict risk of sudden death; and recent genome-wide association studies(GWAS) have revealed common single nucleotide polymorphisms(SNPs) associated with variation in ECG indices of conduction and repolarization. In this study we investigated the effect of these SNPs in modulating risk of VF in acute MI.
Methods: Patients studied were from the **Arrhythmia Genetics in the NEtherlands Study (AGNES),** which consists of Dutch Caucasian patients with a first acute MI, with those suffering VF classified as cases(n=515) and those not suffering VF classified as controls(n=457). SNPs associated with ECG conduction and repolarization indices at genome-wide significant p-values ($P<5\times10^{-8}$) in previous GWAS were identified. Genotypes were obtained either by direct genotyping(Illumina610) or were imputed using HapMap build 36. Logistic regression was used to test for association with VF.
Results: Besides previously reported rs6795970 in *SCN10A*, two SNPs, rs11897119 and rs17779747 were found to modulate risk of VF during MI. rs11897119 (OR:1.23, 95%CI:1.03-1.49) is located in *MEIS1*, and was previously shown to be associated with PR-interval in the general population. rs17779747 (OR:1.28, 95%CI:1.05-1.54) is located 600 kb far from *KCNJ2,* and was previously associated with QT-interval in the general population.
Conclusions: SNPs impacting cardiac conduction and repolarization may modulate risk of sudden cardiac death during acute MI.

P10.70
Incidence of Spinal Muscular Atrophy in Chuvashia

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The proximal spinal muscular atrophy is a severe autosomal recessive neuromuscular disease characterized by degeneration of alpha motor neurons in the spinal cord, which results in progressive proximal muscle weakness and paralysis. This disease is caused by mutation in the telomeric copy of the survival motor neuron gene (*SMNt*). Most carriers of SMA have one chromosome 5 with a normal *SMNt* gene and one with a deleted *SMNt* gene.
This study sought to evaluate the incidence of SMA in Chuvashia.
Multiplex Ligation-dependent Probe Amplification was used to assess the copy number of the two survival motor neuron genes (*SMNt* and *SMNc*) on chromosome 5q13. This method allows to determinate accurately the carriers for spinal muscular atrophy (SMA), with one copy of *SMNt*.
Analysis of 260 normal Chuvashia individuals revealed a carrier incidence of 2,7% (1 per 37, 7/260), the calculated incidence of SMA equal to 1 per 5476. These findings are significant comparable with carrier frequency 1 per 40-50 and incidence 1 per 6000-10000 in the general population.

P10.71
Association of single nucleotide polymorphisms in IL-20 and IL-10 with Hepatitis C virus

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Back ground: Hepatitis C virus (HCV) is an infectious blood-borne pathogen that usually persists as a chronic infection. However, in approximately 15% of cases, patients can clear the virus, indicating that host differences could be critical in determining the course of HCV infection. The inflammatory response is crucial to resolve the virus or progress to chronic infection after acute phase. The aim of this study was to determine effects 5 single nucleotide polymorphisms (SNPs) in the IL10 and IL 20 genes for association with HCV.
Methods: We conducted based case-control study. DNA from 120 patients and 110 controls was investigated. IL-20 gene single nucleotide polymorphisms in (rs1518108), (rs1400986) and IL-10 single nucleotide polymorphisms in (rs1800872) , (rs1800871) and (rs1800896) were evaluated by polymerase chain reaction and the restriction fragment length polymorphism technique.
Result: This study demonstrated a significant association between polymorphism of rs1518108 and HCV infection in the Iranian population. Also no significant differences were found in the polymorphisms of IL-20 and Other IL-10 allele polymorphisms between patients and controls.
Conclusion: The rs1518108 T allele might be a risk factor for HCV in Iranian population.

P10.72
Human chromosome 4p and Malaria Infection

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In the world over 300 million people are infected by malaria each year. In Brazil this cases are concentrated in legal Amazon region. It is known that human genetic factors may contribute to susceptibility and the response to infectious diseases. Genes like HbS, DARC, G6PD and others are responsible for resistance to Malaria infection and the severity of the disease.

Tool-like Receptors (TLRs) are a family of signaling proteins involved in the innate immune response to different kind of pathogens. In the last few years several studies associating TLR genes with infectious diseases were reported.

Recent studies by our group identified a genetic association between the number of malaria infection and a specific region of the human chromosome 4p in Western Amazonian individuals, using a panel of STR markers. It has been postulated that the TLR1 gene may be a good candidate for susceptibility/resistance to malaria infection, since it is related to others infectious diseases and it is located in the 4p human chromosome.

A sample from the Western Amazonian population of Monte Negro (RO) was studied in a Case-Control fashion. Seventy cases and controls were selected through regression analysis, based on the sex, age and the malaria history of 925 individuals. The genotyping assay was made by RFLP of the rs5743618 (I602S).No significant association was detected between the number of malaria infection and the genotype of the analyzed SNP. Other causes for these associations should be searched in order to explain the above findings related to 4p chromosome(CNPq/CAPES).

P10.73
Evaluation the number of carriers of MEFV common mutations in Familial Mediterranean Fever in North-Western area of Iran.

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Background- Familial Mediterranean Fever, one of the most common and recognized periodic fevers syndromes, is a recessive autosomal disorder. This disease is prominently present in the non- Ashkenazi Jews, Arabs, Turks and Armenian people.

Considering the geographic location of North-West of Iran and its neighborhood with two high-risk regions i.e. Armenia and Turkey, the high prevalence of FMF in this area is expected.

Aim- The purpose of this study was to evaluate the number of carriers of MEFV common mutations in healthy controls. The result can be of help in estimating the prevalence of Familial Mediterranean Fever disease and awareness-raising to avoid potential Amiloidosis.

Methods- 200 healthy controls who were not suffering from FMF were selected randomly from North-West area. After obtaining their consent and blood sampling, their DNA was extracted. Then through PCR-RFLP and PCR-ARMS techniques, the mutation was studied.

Findings- Out of 400 studied alleles, 44 mutant alleles for E148Q mutation and 7 mutant alleles for V726A mutation were found. In the other 3 mutations, no mutant allele was found. The frequency of general alleles for these 5 common mutations is 0.1325. The carriers rat was 23.4%.

Conclusions- The result with 23.4% of carriers indicates that FMF is significantly prevalent in North-West of the Iran.

This high number suggests that North-West of Iran, like other high-risk areas, is susceptible to Familial Mediterranean Fever. Like other studies carried out in other countries, this study also suggests that E148Q has reduced influence in the patients of North-West area.

P10.74
Association between SNPs in TNF gene and clinical manifestations of Dengue in Rondonia, Western Brazilian Amazon

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TNF gene showed a significant association with severe forms of infection caused by Dengue virus (Sierra 2010). By an extensive variability of SNPs, it has become essential tool for genetic linkage studies or association in composition and frequency within and between different ethnic groups (Vejbaesya, 2009). Aiming to genotype the SNPs -238 and -308 located in the promoter region of this gene and the genetic association observed between individuals infected with dengue, we collected blood samples from 108 individuals living in Cacoal and Jaru, Rondonia's State. DNA extraction was performed using the protocol described by Higuchi and analysis of samples was by PCR and RFLP, followed by electrophoresis on PAGE 6%. Statistical data were obtained using the program GENEPOP (Version 4.0). The allele frequency was 0.936 for -238G, and 0.0640 for -238A, corresponding to that observed in European populations (0.068 and 0.932 respectively, NCBI, 2010). In dengue patients the presence of this mutant allele-238A is associated with protection (Oliveira 2004). In this study, no association was found (p> 0.05). -308G allele had a frequency of 0.878 and -308A of 0.122, similar to described for African-American (0.123 and 0.877 respectively, NCBI, 2010), confirming the ethnic heterogeneity of the study population. Unable to establish a biological basis of susceptibility and resistance in relation to TNF, even though there is data in the literature (Fernández, 2004). New molecular approaches and statistics will be performed for the association dengue / TNF can be analyzed in order to confirm whether or not the literature.

P10.75
Polymorphisms of thiopurine S-methyltransferase (TPMT) gene in the average Roma and Hungarian population samples

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Thiopurine S-methyltransferase (TPMT) gene polymorphisms are the major determinants of interindividual differences in haematological toxicity of 6-mercaptopurine. The purpose of this work was to determine the frequency of *TPMT* polymorphisms, to study the genetic variability and ethnic differences in average Hungarian, and Roma population samples. Total of 370 Hungarian and 271 Roma subjects were genotyped for the *TPMT*2* (G238C, Ala80Pro, rs1800462), *TPMT*3B* (G460A, Ala154Thr, rs1800460), *TPMT*3C* (A719G, Tyr240Cys, rs1142345) and *TPMT*3A* (G460A and A719G) variant alleles by PCR-RFLP assay. Comparing the genotype and allele frequencies of Roma and Hungarian populations differences were found in the *TPMT* 460 GG (93.0 vs. 96.5%), GA genotypes (7.00 vs. 3.50%) and in A allele frequency (0.035 vs. 0.018) between the two studied groups ($p<0.05$). Futhermore, in frequency of *TPMT* 719 AA (91.5 vs. 95.4%), AG (8.50 vs. 4.60%) genotype and in G allele frequency (0.042 vs. 0.023) was significant difference between the Roma and Hungarian population samples ($p<0.05$). Predominant variant alleles were the *TPMT*3A* (7.00 and 3.52%) and the *TPMT*3C* (0.70 and 0.54%) both in Roma and Hungarian populations. Interestingly, the frequency of total variant alleles was almost two times higher in Roma than in Hungarian population samples (7.70 vs. 4.20%). The results of *TPMT* polymorphisms found in the Hungarian population were similar to that observed in other Caucasian populations. By contrast, the Roma population differs from Hungarians, from Caucasians, and from populations of India in common *TPMT* polymorphisms.

P10.76
Analysis of UCPs, PPARs and PGC-1 genes polymorphism in elderly people

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Our goal was to investigate whether polymorphisms in *UCP2, UCP3, PPARA, PPARD, PPARG* and *PGC-1* genes influence life expectancy. All genes are involved in storage and energy burning and provide opportunity to study energy balance in humans.We have analyzed six polymorphisms of this genes: *UCP2* Ala55Val, *UCP3* C-55T, *PPARA* G/C, *PPARD* +294T/C, *PPARG* Pro12Ala and *PGC-1* Gly482Ser, by RLFP method in 145 elderly people (group1) and 133 control group (25-65 aged people) (group2) from North-West Region of Russia. Distribution of genotypes of *UCP3* gene was significantly different between group1 in comparison to group2 (p=0.009, df=2) for C/C genotype 43.9% and 24.4%, C/T genotype 46.2% and 67.1%, T/T genotype 9.8% and 8.5%, respectively. So as for *PGC-1* (p=0.04, df=2) for Gly/Gly genotype 40.0% and 28.6%, Gly/Ser genotype 47.6% and 62.4%, Ser/Ser genotype 12.4% and 9.0%, respectively. For *PPARD* polymorphism both genotypes and alleles differences were founded between group1 in comparison to group2 (p=0.0007, df=2) for T/T genotype 66.0% and 88.8%, T/C genotype 31.9% and 9.0%, respectively, and increasing of C allele in group1 compared with group2 (18.1% and 6.7%, respectively) and decreasing of T allele in group1 in comparison to group2 (81.9% and 93.3%, respectively). Also increasing of C allele (*PPARA*) in group1 in comparison to group2 (26.3% and 17.5%, respectively, p=0.07) and decreasing of G allele in group1 in comparison to group2 (73.7% and 82.5%, respectively, p=0.07) were founded. Consequently we suggest that *UCP3, PPARD* and *PGC-1* polymorphisms are significant for survival.

P10.77

Vitamin B12 status during pregnancy and cognitive ability of children at age 8: a Mendelian Randomization study in a UK birth cohort

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Vitamin B12 is essential for maintenance of a healthy neuronal system. Brain development occurs primarily in utero and early infancy, but the role of maternal vitamin B12 levels during pregnancy on offspring cognitive function is unclear. In this study we examined the relationship between vitamin B12 status in pregnant women from the UK ALSPAC birth cohort and the cognitive ability of their offspring. First, we conducted an observational study of maternal vitamin B12 dietary intake during pregnancy and child's IQ at 8 years of age. There was a positive observational association between maternal vitamin B12 intake and child's IQ that was attenuated somewhat after adjustment for potential confounders, but remained. We then employed a Mendelian randomization strategy that assessed the association of maternal genotype at SNPs in the genes FUT2 (rs492602) and TCN2 (rs1801198), which have been previously found to be reliably related to serum vitamin B12 in genomewide and candidate gene association studies, with child's IQ. This approach allows us to evaluate the causal relationship between exposure and outcome without confounding from lifestyle and environmental risk factors. In this case, maternal alleles associated with an increase in serum vitamin B12 were also associated with higher IQ scores in children. These associations were present after adjustment for the equivalent offspring SNP and population stratification. Our findings support the hypothesis that low vitamin B12 levels in utero adversely impact children's cognitive function.

P10.78

Allelic determinants of vitamin D insufficiency and bone mineral density

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Vitamin D (25(OH)VitD) plays an important role in many areas of health and disease. Prospective studies showed associations between low vitamin D and mortality. Vitamin D insufficiency affects as many as half of otherwise healthy adults in developed countries. The vitamin D binding protein gene GC ("group-specific component") contains an A>C polymorphism, whereby the GG genotype has been associated with an increased risk for vitamin D insufficiency. We investigated the potential role of this GC polymorphism for bone mineral density and bone fractures.

GC genotypes were determined in two independent cohorts, 370 elderly otherwise healthy and in 970 nursing home subjects. Genotypes were related to 25(OH)VitD and fractures in both groups and with bone mineral density (BMD) in healthy elderly subjects.

Mean vitamin D levels were within the lower normal range in the healthy elderly cohort (32,8±13,2 ng/ml) and very low in the nursing home patients (9,3±6,8 ng/ml). The GG genotype was highly associated with lower 25(OH)VitD values (p<0,001). Age-adjusted measurements of BMD expressed as Z-scores (n=356) at the lumbar spine, the total hip and femoral neck were not associated with the GC polymorphism. GC frequencies were not statistically different between persons with fractures (n=583, GG: 8,2%, GT: 42,7% TT: 49,1%) and persons without fractures (n=744, GG: 3,6%, GT: 41,1% TT: 52,3%).

We conclude that the GC polymorphism is a determinant for raised risk of Vitamin D insufficiency but not for bone mineral density and fracture risk, at least in our highly deficient 25(OH)VitD nursing home cohort.

P10.79

The place of the population of Lithuania between Northern and Eastern Europe: Y chromosome analysis

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The population of Lithuania is constituted of 6 dialectal groups which form two major ethno-linguistic groups known as Aukštaitish and Žemaitish, both speaking Baltic languages of Indo-European family. Neighbouring Finno-Ugric (Northern and Eastern Europe), Slavonic (Eastern Europe) and Germanic (Northern Europe) populations surrounding the Baltic sea region influenced historical formation of Lithuanian ethno-linguistic groups. Analysis of the Lithuanian population genetic composition helps to understand the origin, history and place among other populations.

Y chromosome analysis was performed for 301 individuals from 6 dialectal groups. 25 SNPs were genotyped (TaqMan) to determine Y haplogroup and 17 STR were analysed to determine haplotype for each individual. Most frequent haplogroups in the population of Lithuania are R1a1a (42.2%, R1a1a1g compose 8.97% in studied population) and N1c1 (40.5%) and less frequent haplogroups are R1b1b1, I1, I2a, E1b1b1 (<5% each). AMOVA showed no statistically significant differences between two major ethno-linguistic groups Aukštaitish and Žemaitish (among groups p-value=0.897, among population within groups p-value=0.194, within populations p-value=0.282 based on 10100 permutations). MDS of genetic distances based on Y-biallelic markers showed that Lithuanians are closer to Latvian and Estonian populations than to Slavic populations (European part of Russia, Poland, Ukraine, Belorussia, stress=0.029).

According to the frequencies of haplogroups, no statistically significant differences between ethno-linguistic groups were detected (p>0.05), moreover, MDS analysis sets the population of Lithuania between Northern and Eastern European populations.

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P10.80

Y-Chromosome genetic variation of modern Bulgarians

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To date, Bulgarian Y chromosomes have been studied only in macrogeographic context or in the lineage-based approach. Therefore, in order to comprehensively characterize Bulgarian Y-chromosome variation, we have performed high-resolution phylogenetic analysis of 812 healthy,unrelated Bulgarian males and compared the results with Y-chromosome data from other Eurasian populations.

The genotyping of 60 biallelic markers was performed in hierarchical order by RFLP and DHPLC analyses. The position of Bulgarians among other populations was visualized by Principal Component (PC) analysis.

About 80% of the total genetic variation in Bulgarians falls within haplogroups E-M35, I-M170, J-M172, R-M17 and R-M269. This finding shows that the Bulgarian haplogroup profile is congruent with those described for most European populations.

Among the prehistoric events marked by the observed haplogroups, the greatest contribution comes from the range expansion of local Mesolithic foragers triggered by adoption of agriculture introduced by a cadre of Near Eastern farmers. The Bulgarian Y chromosome gene pool also bears signals of the recolonization from different glacial refugia, the spread of agriculture from the Near East and the expansion of early farmers along the Central and East European river basins.

As for the interpopulation analysis, similarly to mtDNA, Bulgarians belong to the cluster of European populations, still being slightly distant from them. Bulgarians are distant from Turks (despite geographical proximity), Arabic and Caucasus populations and Indians. These trends in the PCA graph likely reflect not only prehistoric, but also more recent demographic events that have shaped the Y chromosome structure of modern Bulgarians.

P10.81

Eight-year investigation of Yq-microdeletions in azoospermic and severe oligozoospermic men in Iranian population

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Genes on the long arm of Y-chromosome (Yq), particularly within interval 6, are believed to play a critical role in human spermatogenesis. It has shown that microdeletions of Yq may account for a significant proportion of men with infertility in different populations. Y-chromosome microdeletions are known as the second frequent genetic cause of spermatogenetic failure in infertile men after Klinefelter syndrome.

Over last 8 years, total of 900 infertile individuals referred to our center for Yq-microdeletion analysis. The aim of this study was to determine the proportion of men with idiopathic azoospermia or severe oligozoospermia, who carry microdeletions in Y-q.

EDTA blood was taken from each of patients and DNA extracted according to standard protocol. Yq-microdeletion analysis was performed by PCR method using different STSs multiplex reaction designed for covering three regions of Y-chromosome known as AZFa, AZFb, and AZFc. As the results showed, 4.7% azoospermic and oligozoospermic samples failed to amplify 1 or more STS. Interestingly, 34 out of 42 patients had microdeletions in AZFc region that accounts for 80.95% of all detected microdeletions.

These data suggest a 4.7% prevalence of Yq-microdeletions in Iranian men with idiopathic azoospermia/severe oligozoospermia. The physical locations of these microdeletions provide further support for the concept that a gene(s) on Y-q interval 6 plays an important role in spermatogenesis.

P10.82

A gene involved in metabolism modulates natural variation in human sleep duration

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Extremes of sleep duration and timing have been associated with adverse health outcomes that characterise the metabolic syndrome. Body homeostasis and circadian rhythm are though to interact to regulate these phenotypes, but little is known about the molecular mechanism behind it. We thus investigated 7 European populations in relation to their sleep duration habits and genome-wide variability. Meta-analysing the independent genome-wide association results, we identified a variant (P = 3.99 x 10^{-8}) in the *ABCC9* gene that explains ≈ 5 % of the variation in sleep duration. We found supportive evidence for this association in a subgroup of an independent *de novo* replication cohort, which we selected based on gene-environment (time of year) and sleep timing interactions. To investigate the functional relevance of our findings, we knocked down a homologue of the gene in *Drosophila,* which resulted in a night-sleep duration reduction of 3 h. Our study shows that *ABCC9* modulates epidemiological variation in human sleep duration, which is also influenced by inter-individual differences in sleep timing and seasonality (seasonal differences in entrainment of the biological clock). Therefore, scanning only for main effects on sleep duration, one might miss important genetic variants specific to subgroups of the population. *ABCC9* is involved with energy homeostasis, and the susceptibility to overweight and cardiovascular disease, which correlate with sleep duration. The relation of these genes to metabolism and disease indicates a common mechanism for the regulation of these phenotypes and sleep duration.

J10.01

HPV infection prevalence and subtypes‘ distribution among women with cytology findings, attending two private clinics in Larissa (central Greece)

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INTRODUCTION: Infection from Human Papillomavirus has been establisthed as a cause of cervical cancer. HPV screening in women with positive cytology results, helps to identify the women in need for therapy and further screening. The aim of the study was to identify the most prevalent HPV subtypes in Larissa, Greece. The sample population consisted of women attending two private gynaecology clinics and referred to our laboratory for HPV screening due to findings in their cytology test indicating HPV infection.

METHODS: A total of 255 women were studied. All women underwent a regular gynaecological examination including Papanicolaou test. None of the women had been previously diagnosed with an HPV infection. The following 32 HPV types were studied using a commercially available method based on an LCD array hybridization (CHIPRON), following a duplex PCR :

6, 11,16,18,31,33,35,39,42,44,45,51,52,53,54,56,58,59,61,62,66,67,68,70,72,73,81, 82,83,84,90 and 91.

RESULTS: HPV infection was detected in 79.3% of the study population. Among the infected population, 35.8% had a single type

infection with the most prevalent type being type 16 (18%) followed by types 45 (9.7%) and 56 (8.3%).
22% of the infected population had infection with 2 types of hpv, 17% with 3 types, 13,9% 4 types, 5.4% 5 types. The rest had between 7 and 16 types of HPV.
CONCLUSIONS: There is a high prevalence of HPV infection amongst women attending private gynaecology clinics in Larissa. Findings are in accordance with the published literature on the prevalence of each subtype studied.

J10.02
Population Genetic Study of Russia

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Parameters of Barrai calculated in populations: 13 districts of Rostov region (I_r from 0.0005 to 0.0022) , 29 districts of Kirov region (I_r from 0.0017 to 0.0201), 6 districts of Tver region (I_r from 0.0031 to 0.0049), 2 districts of Arkhangelsk region (I_r from 0.0020 to 0.0030), 7 districts of Marii El Republic (I_r from 0.0017 to 0.0133), 33 districts of Yakutia (Sakha) Republic (I_r from 0.0010 to 0.0468), 16 districts of Tatarstan Republic (I_r from 0.0008 to 0.0065). Parameters of Isolation by distance of Malecot calculated in populations: 6 districts of Chuvashia Republic (a from 0.00016 to 0.00054), 5 districts of Marii El Republic (a from 0.00025 to 0.00058), 2 districts of Yakutia (Sakha) Republic (a from 0.0013 to 0.0106), 10 districts of Rostov region (a from 0.00012 to 0.00046), 7 districts of Kirov region (a from 0.00011 to 0.00149), 2 districts of Tver region (a from 0.0009 to 0.0011), 2 districts of Arkhangelsk region (a from 0.00047 to 0.00056), 6 districts of Bashkortostan Republic (a from 0.00015 to 0.00227), 6 districts of Udmurtia Republic (a from 0.00027 to 0.00065), 12 districts of Tatarstan Republic (a from 0.00012 to 0.00041). Crow Index and its components calculated in rural populations: Rostov region (I_m=0.050, I_f=0.211, I_{tot}=0.272), Tver region (I_m=0.029, I_f=0.171, I_{tot}=0.205), Pskov region (I_m=0.019, I_f=0.261, I_{tot}=0.286), Marii El Republic (I_m=0.105, I_f=0.424, I_{tot}=0.575), Chuvashia Republic (I_m=0.068, I_f=0.273, I_{tot}=0.360), Yakutia (Sakha) Republic (I_m=0.072, I_f=0.364, I_{tot}=0.462), Bashkortostan Republic (I_m=0.051, I_f=0.251, I_{tot}=0.315), Udmurtia Republic (I_m=0.046, I_f=0.258, I_{tot}=0.316), Kalmykia Republic (I_m=0.038, I_f=0.300, I_{tot}=0.350).

J10.03
Genetical and epidemiological research of a hereditary ophthalmic pathology among the children‘s population

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We carried out genetic and epidemiological research into hereditary ophthalmic pathology (HOP) among children of 9 districts (Bogorodsky, Svechinsky, Nemsky, Uninsky, Shabalinsky, Darovsky, Verhoshizhemsky, Kumensky, Sunsky) of the Kirov province (Russian Federation). The total investigated population was 93,790, of whom 17% were children (0-18 years old inclusive). The prevalence of HOP among children was 2.31±0.38 per 1000 persons. The ophthalmologist in one of the districts actively carried out routine inspections, starting at 2-3 years old. This method allowed detection and registration of disease in an initial stage of illness, without the presentation of subjective complaints, and subsequent monitoring. Considering the high prevalence of isolated HOP, the pronounced clinical variability, genetic heterogeneity, severity of the pathological processes, and the extent of disability in the population, it will be necessary to carry out routine inspections of children. Early diagnosis of disease will help not only to reveal cases, but it will also motivate treatment of illnesses at sub-clinical levels of the pathological process, that considerably postpone long-term disability. It will raise the quality of life and will positively impact on social and economic welfare.

P11 Genomics, Genomic technology including bioinformatics methods, gene structure and gene product function and Epigenetics

P11.001
Investigation of an atypical 16p11.2 microdeletion as a possible pathogenic rearrangement associated with congenital defects and mental retardation.

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Chromosome 16p11.2 deletion is an emerging syndrome associated to a variable phenotype, including autism. The common deleted region extends from genomic location 29.4-31.1Mb and its flanked by low copy repeats. A deletion of the adjacent region has been also described in a boy with mental retardation and his father with learning disabilities. We present an additional case with the same atypical 16p11.2 deletion and speculate about its possible pathogenic effect.
The girl showed facial dysmorphic features, congenital malformations (aortic valve defects, agenesis of the corpus callosum and uretero-vesical reflux), moderate-severe mental retardation and speech disorder. She carried a *de novo* balanced (1;17)(q21;q23) translocation, observed by conventional karyotype. Genome-wide oligonucleotide arrays studies detected a 289kb deletion (location 28.73-29.02Mb), flanking the recurrent 16p11.2 microdeletion/duplication region. The deletion was confirmed by FISH studies and ruled out in both parents. Translocation breakpoints, located at 1q11.1 (a non-containing gene region) and 17q23.1.q23.2, did not present genomic imbalances. It has been shown that around 30% of cases with apparently balanced reciprocal translocations and abnormal phenotype present genomic rearrangements in other chromosomes. We, therefore, hypothesis that the atypical deletion of 16p11.2, present in our patient and previously reported in two cases with intellectual disabilities, may have a pathogenic effect, either as a primary cause or as a contributing factor to the phenotype. Further investigations to rule out the possibility of gene disruption within 17q23 region and characterization of additional cases with 16p11.2 deletions are needed to fully understand the role of this rearrangement.

P11.002
Study of the ACVR1 gene expression and regulation: the 5‘UTR and the promoter region

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ACVR1 encodes a BMP type I receptor mutated in Fibrodysplasia Ossificans Progressiva, a severe form of heterotopic ossification. Mechanisms regulating ACVR1 expression are still unknown. According to our analysis, the 5’ genomic region of ACVR1 is subjected to alternative splicing generating several transcripts in which different 5’UTR exons are combined to a common coding sequence, with different expression profiles according to cell type. As assessed by cell transfection with different full length cDNA constructs and western blot analysis, we observed that different splicing isoforms correlated with different quantitative expression of the protein.
Moreover, with the aim to identify regulatory elements involved in the transcriptional control of ACVR1 expression, we focused on a genomic region spanning around 2.9 kb, from position -2.893 to +32 upstream of transcription start site (tss, +1). This region is highly conserved and particularly rich in GC nucleotides. A functional characterization of the ACVR1 promoter was obtained by subcloning the whole fragment and a series of derived deletion segments in a Luciferase expression vector and transfection in different cell lines. The strongest promoter activity was displayed by a 700 bp fragment upstream of tss, but a residual and quite significant activity was still present in a 100 bp fragment.
Our data suggest that besides the transcriptional control of ACVR1 expression operated by the promoter region, other mechanisms involving the 5’UTR region, in particular translational regulation, can take place.
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P11.003
Identification of new putative Alpha globin mutations in Iranian population

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Alpha thalassemia is usually caused by deletions in alpha globin gene cluster, and the role of point mutations is less investigated.
In present study a total of 3438 individuals with unexplained hypochromic microcytic anemia who did not reveal the most common alpha thalassemia deletions (3.7kb, Med, 4.2kb, 20.5kb) by gap-PCR, were subjected for alpha2 and alpha1 globin gene DNA sequencing, respectively. Total of 2194 alpha2 and 1244 alpha1 globin DNA sequencing assays were performed. In cases of absence of nucleotide change, the sequences were analyzed by CodonCode aligner software (V. 3.7.1), to exclude frame shifts and none sense mutations. Other variations were checked by Polyphen online software to identify whether the mutation is damaging or not.
Seven new mutations have been identified, of which 2 located in alpha2 loci (Cd 83 T>G, Cd43 C>A) and 5 in alpha1 loci (Cd99 A>T, Cd34 T>C, IVSI-4 A>G, Cd44 insC and Cd109 delC). Among alpha1 globin mutations, the Cd99 was nonsense, Cd44 and Cd109 were frameshifts, and the Cd34 was considered as possibly damaging by Polyphen but the role of IVSI-4 needs to be further analyzed.
This study remarks the importance of point mutations in alpha globin gene cluster, and can identify their role in microcytic anemia. Besides, there were plenty of nucleotide variations identified as polymorphisms. Locating these positions and studying their relations with damaging mutations enables us to perform a population study for discovering the origin of the mutations.

P11.004
Rapid high-throughput Alport diagnosis by next generation sequencing.

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Alport syndrome is an hereditary nephropathy often associated with sensorineural hypoacusis and ocular abnormalities. Mutations in the *COL4A5* gene causes X-linked Alport syndrome. Mutations in *COL4A4* and *COL4A3* genes have been reported in both autosomal recessive and autosominal dominant Alport syndrome. The conventional mutation screening, performed by DHPLC and/or Sanger sequencing, is time consuming and has relatively high costs due to the absence of hot spots and to the high number of exons per gene: 51 (*COL4A5*), 48 (*COL4A4*), and 52 (*COL4A3*). Usually, several months are necessary to complete the diagnosis, especially in cases with less informative pedigrees. To overcome these limitations, we designed a next generation sequencing protocol enabling simultaneous detection of all possible variants in the three genes. We used a method coupling selective amplification to the 454 Roche DNA-sequencing platform (GS junior). The application of this technology allowed to identify the second mutation in two Alport patients (p.S1147F in *COL4A3* and p.R1682W in *COL4A4*) and to exclude the diagnosis of Alport in a third patient. This study therefore illustrates the successful application of next generation sequencing to routinely diagnosis of Mendelian disorders with locus heterogeneity.

P11.005
Genotype-phenotype correlation in X-Linked Alport syndrome patients carrying missense mutations in the collagenous domain of COL4A5

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The X-linked Alport syndrome (XLAS) is caused by mutations in *COL4A5*. More than 400 mutations have been reported in the literature to date and this allelic heterogeneity is also reflected by a phenotypic heterogeneity. Genotype-phenotype correlation remains elusive, although recent analyses have shown that missense mutations (of which the majority are glycine substitutions) give a better prognosis than other types of mutations. A genotype-phenotype correlation was examined with regards to age at onset of ESRD and: (i) distance of mutation from closest natural interruption, (ii) location of mutation along the gene, (iii) change in number of side-chain carbon atoms caused by the mutation, and (iv) change in charge and hydrophobicity caused by the mutation. Trends were evident for all statistical analyses and a statistically significant (p-value: 0.0017) correlation (R square: 0.1362) could be found, with age at onset of ESRD decreasing with increasing number of carbon atoms found in the side chain of the substituting amino acid. We also examined the possibility of interruptions representing subtle mutational hotspots and although a trend could be seen with having less mutations within interruptions, no statistical significance could be reached. Hence, the size of the side chain of the substituting amino acid is an important factor in determining the disease phenotype with regards to age at onset of ESRD. This can be explained by the fact that any other aminoacid substituting glycine is larger and therefore cannot be accommodated where the three chains meet in the collagenous triple helix.

P11.006
Targeted Re-Sequencing in ALS / FTD and patients with neurodegenerative and movement disorders

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Background: Several genes have been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) and other types of neurodegeneration. As each gene accounts for only a small fraction of cases, it is crucial to develop an efficient tool for investigating all genes simultaneously. Here we present a very fast and cost-efficient targeted re-sequencing approach to screen for all known ALS / FTD and genes associated with neurodegeneration and potential candidate genes simultaneously. We expect to identify major genetic risk factors in a significantly larger proportion of cases as compared to the cases identified with the conventional sequencing method. In addition, careful collection of all clinically relevant information will allow us to investigate how the genotype determines the phenotype in the different subsets of neurodegenerative diseases.
Methods: Genomic DNA is enriched using a custom made Agilent SureSelect in solution kit. Sequencing of 50 genes (360kb in total) is performed using barcoded libraries on one oct (1/8 slide) on the SOLiD 4 platform generating approximately 5 Million mappable 50 basepair reads per patient. The basic data analysis is performed with Bioscope v1.2. Additionally, we developed a diagnostic pipeline. All variants are then re-sequenced by the gold standard Sanger sequencing or quantitative PCR, respectively.
Results and Conclusion: We introduce a fast and highly efficient screening tool for variants in genes associated with movement disorders and neurodegenerative diseases. Here, we present solved cases and troubleshooting of unsolved cases.

P11.007
Clinical significance of rare CNVs in epilepsy: a case-control genome-wide study

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This study show an extensive search for genomic rearrangements by microarray-based comparative genomic hybridization (array-CGH) in patients with epilepsy of unknown etiology.

Two sets of 279 patients with unexplained epilepsy and 246 controls were screened by using a 44K CGH-array. CNVs overlapping >50% with those reported in the Database of Genomic Variant were not followed-up. Validation was performed by FISH, higher density array-CGH, or quantitative-PCR. GeneCodis2.0 software was used to explore the biological function of genes included within the identified CNVs.

Rare CNVs occurred in twenty-six (9.3%) patients and in sixteen (6.5%) controls (p=0.26). CNVs identified in patients were larger (p=0.027) and showed higher gene content (p=0.021) than controls. CNVs sized >1Mb (p=0.002) and including >10 genes (p=0.005) occurred more frequently in patients than controls. Conversely, the two groups did not differ for CNVs smaller than 1Mb (p=0.051) and involving 10 or less genes (p=0.547). Nine (34.6%) patients among those harboring rare CNVs showed rearrangements associated with emerging microdeletion/microduplication syndromes. Mental retardation and neuropsychiatric features were associated with rare CNVs (p=0.004) whereas epilepsy type did not. Significant enrichment of genes involved in ion transport was observed within CNVs identified in patients. Patients with epilepsy have a significantly increased burden of large, rare, gene-rich CNVs, particularly when associated to mental retardation or other neuropsychiatric features. Therefore, we suggest that CNVs screening should be routinely performed for diagnostic purposes only in such patients. Moreover, the implementation of very high resolution screening in clinical practice deserves caution.

P11.008

Assessing size distribution and integrity of high molecular weight DNA prior to aCGH experiments

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Multiple genomic applications such as for example aCGH or next-gen sequencing (NGS) require highest DNA quality as the level of DNA degradation can have a significant effect on downstream sample processing and therewith final data quality and interpretation. Over the past years, microfluidic on-chip electrophoresis has become the standard tool for assessing size, quantity and integrity of DNA and RNA starting material in many experimental workflows. With an optimized protocol and improved electrophoresis chemistry, the analytical size range could be extended which resulted in a new prototype assay. This extended size range enables the integrity assessment of high molecular weight DNA isolated from tissues or cell lines by column based extraction methods. In contrast to slab gel analyses, this prototype assay provides standardized information on DNA size distribution, quantity and integrity. Results from a study correlating DNA sample quality with aCGH read-outs are discussed.

P11.009

Diagnostic use of targeted array CGH platforms: 2009-2010 Kocaeli University Experience

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INTRODUCTION: Array CGH technology is a new method for screening human genetic disorders that cause changes in DNA copy number. This molecular karyotyping technology is remarkable with fast analysis and highly sensitive diagnostic value. We aimed to use this technology as a diagnostic tool in Kocaeli University Hospital. We performed analysis for nine groups of patients, using different targeted array CGH platforms.

MATERIALS and METHOD: Following patient groups were accepted to Kocaeli University Medical Genetics Department; Microdeletion Syndrome(35 patients) Primary Autism(35 patients), 18q21.31 Deletion Syndrome(2 patients), Mental Retardation(52 patients), Prenatal Diagnosis(10 patients), Implantation Failure(50 patients), Primary Amenorrhea(1 patients), IVF improvement(7 patients) and Hematological Malignacy(4 patients). We isolated DNA samples of 514 patients. DNA samples labelled according to the protocols.Samples were hybridized with CytoSure Syndrome Plus(v2) 4x44K microchips and with 24Sure platforms.Hybridised platformes were scanned with Agilent Microarray Scanner.Scanned results were analysed by Cytosure Analysis Software-v.2.0.8 and BlueFuse Multi-v2.1.

RESULTS: We found 7 aberrations in 35 autistic patients, 35 microdeletions in 35 patients, 6 aberrations in 10 prenatal diagnosis investigations, 38 aberrations in 52 mental retardation patients, 4 aberrations in 50 implantation failure patients, and 5 aberrations in 4 hematological malignancy patients.All aberrations were confirmed with FISH analysis.All analysis have been completed less then 72 hours. Starting DNA amounts were between 10ng to 500ng.

CONCLUSION: We conclude that, targeted aCGH platforms have a precious contribution to solve our diagnostic problems in clinical use. This technology is an efficient tool for delineating chromosomal aberrations. It is an indispensable attachment to conventional cytogenetics.

P11.010

Saudi Biobank experience in enhancing biological and genomic research at King Abdullah international medical research Center

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Biobanks are a new frontier for biomolecular research, clinical genomics and personalized medicine that seeks to integrate collections of bio-specimens with corresponding patient's medical histories, and lifestyle information. Combining and comparing biological tissue samples with genetic and historical patient information, researchers will be able to investigate the fundamental mechanisms of diseases in rich new ways. New insights into molecular and genetic process will lead to better techniques for predicting disease susceptibility, as well as to more targeted and innovative ways to treat serious diseases.

Saudi Biobanking study is designed as a longitudinal study of constitutional and environmental factors influencing the development of most prevalent chronic diseases in Saudi population.

To ensure the value of the stored biological material in serving is main purpose of advancing healthcare of our country through research. Ethical standard and Several quality measures has been implemented to guide the collection process, sample processing, health data acquisition, blinding and retrieving the Saudi Biobank (SB) is designed to store for more than 4 million tubes from 200,000 persons. The blood components, urine, tissue DNA, RNA and dry DNA/blood materials will be accumulate into SB. In addition to that, the banking of purified DNA/RNA from patients will not be limited to the above number; however, DNA and Buffy coat is already in our bank.

This presentation is to summarize the value of biobanking in enhancing biological and genomic research.

P11.011

BioSHaRE-IT: Solving the bioinformatic challenges of biobanking

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Current IT infrastructure in biobanking is imperfect. Biobank databases are often rather basic and almost never interoperable. The project 'Biobank Standardisation and harmonisation for Research Excellence in the European Union' (BioShaRE-EU http://bioshare.eu/) was launched in early 2011. It explicitly addresses the informatics needs of biobanking through its sub-program 'BioSHaRE-IT'.

BioSHaRE-IT will work with other initiatives (e.g., BBMRI, DataSHaPER, ELIXIR, GEN2PHEN, OBiBa, P3G) as part of an open collaboration, to tackle many complex computational and data management challenges

relevant to biobanking. Efforts will exploit 'starting reagents' such as OPAL, MOLGENIS, GWAS Central (HGVbaseG2P), DataShield, DataShaper, etc, with a focus upon;
1) developing data models for core molecular, phenotype, and environmental data along with ontologies so that data can be managed, integrated and represented optimally
2) developing federated databases for local storage and deployment of different types of biobank content, such as sample and research catalogues, genetic/genomic data, detailed phenotype information, epidemiological and environmental data
3) devising data exchange formats and data access mechanisms (based upon digital identifiers) to allow a smooth and secure exchange of data to, from, and between biobanks
4) providing tools and solutions for study design, phenotype harmonisation, data curation, and data visualisation, so that biobanks can adopt these solutions directly and not have to reinvent them
5) developing tools for biobank data searching and remote analysis, along with distributed grid technologies to deal with the data computation and data storage management issues.
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P11.012
Identifying knowledge contributors - motivating online sharing of research data

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Contributor identification is a core challenge in data publication. As in scholarly communication more generally, non-unique person names and the current lack of a global identification infrastructure for producers of scholarly content makes it difficult to establish the identity of authors and other contributors. This in turn makes it difficult to accurately attribute datasets published via online digital repositories to their creators - one of several key requirements for including these important outputs in the scholarly record.
In the GEN2PHEN project (http://www.gen2phen.org) we are developing a series of novel web-based systems and processes for online dissemination of genetic variation and other research data. The core aim is that of ensuring that data creators are recognized and rewarded for publishing data. This work builds on and integrates with recently launched international initiatives to i) extend and adapt the existing DOI infrastructure for identifying, locating and citing online datasets (DataCite: http://www.datacite.org), and ii) create a global registry of unique identifiers for authors and other contributors (ORCID: http://www.orcid.org).
The technical approach we are exploring in this pilot project utilizes this emerging global data citation and contributor identification framework, in order to allow published datasets to be discovered, cited in a scholarly context and unambiguously attributed. We argue that, along with other measures, such an incentive-based approach is key to motivating the sharing of data and other types of digital research outputs in the life sciences.
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P11.013
HUGETOOL: A computer-based method for automated identification of gene-environment interactions in human studies including a large number of participants, SNPs and environmental factors

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Common diseases are determined by the complex interplay between environmental and genetic factors. However, current methods of data analysis of gene-environment interactions using traditional approaches are inefficient. High-throughput methods of analysis in large scale epidemiological studies including thousands of subjects and hundreds of SNPs and environmental factors should be implemented. We developed an integrative computer tool, HUGETOOL (HUman Gene Environmental inTeraction tOOL), for large-scale analysis of gene-environment interactions, in human studies of complex diseases including a large number of subjects, SNPs, as well as environmental factors. That resource uses standard statistical packages to build and fit the gene-environment interaction models by means of syntax scripts in predicting one or more continuous or dichotomic phenotypes. Codominant, dominant and recessive genetic interaction models including control for covariates are automatically created for each SNP in order to test the best model. Environment variables can be used both as continuous and as categorical. From the standard outputs, HUGETOOL extracts a selected set of parameters (regression coefficients, p-values, adjusted means, etc.), and groups them in a single MS Excel Spreadsheet. The tool allows editing the set of filter parameters, filtering the selected results depending on p-values, as well as plotting the selected gene-environment interactions to check consistency. We implemented our tool in real data obtained in two standard cardiovascular studies carried out in the Mediterranean population, and demonstrated the excellent performance of this tool. In conclusion, HUGETOOL is a very useful and friendly tool for exploring and identifying gene*environment interactions in complex diseases.

P11.014
Evaluation of a novel, single molecule, amplification-free method for the detection of Copy Number Variation (CNV)

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Copy number Variation (CNV) is a major component of the genomic diversity present within the human population. Several methods for genome-wide discovery of structural variants now exist , including array-based genomic hybridization and High throughput SNP genotyping arrays (i.e. GWAS arrays). More recently, structural variation has also been inferred from Second Generation Sequencing data . As CNV calls made from each of these approaches can be subject to appreciable false positive rates, detected variants of interest are generally corroborated by an independent method. The method of choice for CNV confirmation has primarily been real-time qPCR. Here we report the evalution of a novel CNV assay based on the NanoString nCounter Analysis System. The nCounter system uses a hybridization-based single molecule capture approach not requiring amplification of input DNA. Invidual tripartite complexes of the target molecule, a biotin labeled probe and a fluorescently labeled ("barcoded") probe are directly counted. We compared the performance of the nCounter system at 30 putative CNV loci in a set of 96 human samples, all of which have data at these same loci from ABI Taqman CNV assays. A subset of samples also have data available from Agilent aCGH 244k custom and Illumina 1M Infinium arrays. Preliminary results show that the Nanostring method exibits 86% agreement with the Taqman CNV assays across all studied loci and individuals. When three problematic repetitive loci are excluded, concordance rises to 96%. Our initial results suggest the nCounter system will provide a promising alternative for validation of copy number variants.

P11.015
Selection of SNPs to be replicated in GWA investigations: a Bayesian approach

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In GWA investigations, SNPs for replication are selected based either on discovery *p*-values alone or with some consideration of the SNP/gene function. In the former approach, a SNP located within a biologically relevant gene has the same *a priori* probability of being selected as a SNP located in any random genome position. In the latter, inclusion of biological considerations is usually made in a very subjective way. We are developing a Bayesian approach to SNP selection that formally incorporates evidence from different sources supporting the effect of a SNP/gene. We identified 15 sources of evidence, from functional to linkage and association studies, information on which is retrieved from publicly available databases using an *ad-hoc* bioinformatics tool. Since different types of evidence may carry different "weight" in influencing SNP selection, we defined such "weights" through expert-opinion elicitation, using a structured group communication process organised in two Delphi rounds involving 10 anonymous experts. Using as an example a GWA meta-analysis of 20 studies on glomerular filtration rate from the CKDGen consortium (Köttgen et al, *Nat Genet* 2010), we are testing the hypothesis that our approach may help identify true associations in situations with limited sample size. We present the framework of our approach, the types of evidence incorporated in the Bayesian model with "weights" defined by the experts, the bioinformatics tool for evidence retrieval, and preliminary results on the performance of the method.

P11.016

GWAS Central curation tool: solving the bioinformatics challenges of complex data management with MOLGENIS

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The creation, management and validation of complex biological datasets are time-consuming and error-prone, so software tools are needed to ease the burden on curators. We have therefore developed a curation tool in the GEN2PHEN project (http://www.gen2phen.org) for the genetic association database GWAS Central (http://www.gwascentral.org) using the MOLGENIS tool (http://www.molgenis.org) for rapid development of biomedical database software.

GWAS Central is a comprehensive resource of summary-level findings from >700 genome-wide association studies with >18M p-values. Previous methods for importing study metadata and marker data involved time-consuming construction of custom software pipelines. To solve this, we built a dedicated curation tool with many useful features: a 'quick-add' interface for new studies (to become a submission tool for users to submit data directly to GWAS Central); automatic assignment of stable identifiers for all study components; study 'overview' and 'preview' interfaces for quick data management; a PubMed look-up service for annotating studies with citations; and marker data import and validation. We are now constructing a security layer to track updates and deletions within the database.

We are also exploring the use of the SOAP and REST web services generated by MOLGENIS to develop Taverna workflows, which can then be integrated within the GWAS Central site. This will enable users to enact complex queries over millions of p-values from behind a simple, easy-to-use web portal, without requiring significant bioinformatics expertise.

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P11.017

GWAS Central: an advanced database for the integration and comparative interrogation of genome-wide association study datasets

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Comprehensive genome-wide association study (GWAS) data-sets are rarely published in journals or databases, and in the case of negative findings often not reported at all. Consequently, comparing different studies is difficult, and it is impossible to examine a full and unbiased picture of all the data that exist.

To address this deficit, GWAS Central (www.gwascentral.org) (previously HGVbaseG2P) was constructed - representing a free and open access resource for the interrogation of summary-level GWAS data, ultimately combining the features of a database and a scientific journal.

GWAS Central employs powerful graphical and text based data presentation methods for discovery, visualisation and co-examination of many studies, at genome-wide and region-specific levels. Studies of interest can be identified using chromosomal regions/genes and markers. There is also the facility for researchers to securely view their own uploaded datasets alongside many published studies.

Current content includes top p-values from collections; supplementary data; direct researcher submissions; and publicly available data. Consequently, the database now hosts >21 million p-values and 708 studies (vs 3,948 p-values and 798 studies in the NHGRI GWAS catalog), representing an estimated ~5% of all such data yet produced.

A version of GWAS Central will soon be released for research groups to install on their own servers allowing them to manage, analyse and control access to their own data. These installations will be interoperable and searchable as a federated network.

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P11.018

Non-Disjunction: from a Bioinformatic and Epigenetic Approach towards a clinical validation

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The aim of this work is to present a fully "in silico" approach for the identification of genes that might be involved in the susceptibility for non-disjunction diseases and their regulation by methylation processes.

We have carried out a strategy based on the use of online available bioinformatics databases. Among these the most relevant ones used were OMIM, HUGO, GeneOntology and PubMed. Bioinformatics programs were used to select, retrieve and analyse the genes and their promoters to identify regions susceptible of being methylated, and thus identify those genes susceptible of being epigenetically regulated.

As result we have obtained 34 putative susceptibility genes regulated by methylation processes. We were neither on the need of developing new software nor carrying out clinical laboratory experiments for the identification of these genes. So it would be interesting to analyze the methylation patterns of these genes in the mothers of children with Down syndrome (cases) to compare it with the one of mothers of sound children. MSP (Methylation Specific PCR) is a good method to analyze it.

We consider that this "in silico" methodology is robust enough to provide candidate genes that must be checked "in vivo" due to the clinical relevance of non-disjunction diseases with the aim of providing new tools and criteria for their diagnosis. The molecular origin of constitutional aneuploidies is a classical area of research that could be of great interest if investigated with new approaches: bioinformatics and epigenetics.

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P11.019***

Using GENCODE annotation to investigate the functional effects of Loss of Function variants identified by the 1000 Genomes Project

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The GENCODE consortium is producing a reference human gene set rich in alternative splicing and functional annotation that will provide the basis for analysis by the 1000 Genomes project. Manual annotation is supplemented by automated gene predictions from Ensembl to produce the GENCODE gene set, the default annotation in the Ensembl browser. A catalogue of variants in protein coding genes predicted to result in Loss of Function (LOF), based on 1000 genomes data, were identified by investigating the intersection of SNPs and indels from the three pilot phases, together with GENCODE gene models. We have manually analysed 885 SNPs/indels that introduce premature termination codons or splice site disruptions, firstly to validate and ensure completeness of the gene models on which LOF calls are based. One third of all variants investigated were miscalled due to errors in genome sequence or computational gene predictions. Secondly, we have assessed whether the exon containing a LOF variant is constitutive or alternatively spliced, and finally we predict the functional impact of the variant. Of the valid calls, 36% affected exons that showed transcriptional evidence of being skipped in splice variants. Furthermore, 17% are predicted to effect changes in the protein product that may not result in LOF; producing for example small CDS truncations. We will describe how high quality annotation of gene models are essential to accurately predict the consequences of a SNP, and we propose a model whereby LOF may be considered at the level of the transcript and not the overall locus.

P11.020

Automated nucleic acid purification with Thermo Scientific KingFisher magnetic particle processors and KingFisher Kits

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The pure and intact DNA and RNA are doubtless the most important sample materials for various experiments in molecular biology. The magnetic particle technology combines the speed and efficiency of automation with high quality nucleic acid purification capability. Thermo Scientific KingFisher processors offer a patented technology based on the use of magnetic rods with specially designed plastic consumables, and the process can be combined with optimized KingFisher Kits. This automated nucleic acid purification method is described in the poster. Additionally, the process is compared with the competitors' purification systems.

KingFisher Kits are available for genomic and viral nucleic acid purification from wide variety of sample types e.g. blood, cells and animal or plant tissues. We analysed the performance of the purification process with five different KingFisher Kits. The KingFisher Blood DNA Kit was compared to three competitive methods or instruments, and the KingFisher Plant DNA Kit to four competitors. In each case the KingFisher Kits performed as well as or better than the competitors, and the performance was excellent. With the KingFisher Cell and Tissue DNA Kit and Total RNA Kit different sample materials and variable lysis times were tested. The results indicate the sensitivity of the purification process. In addition the performance of KingFisher Viral NA was tested in different applications.

The KingFisher processors together with the KingFisher nucleic acid purification kits and the Thermo Scientific BindIt software constitute an exceptional purification system for obtaining high yield and purity of DNA and RNA.

P11.021***

Integrative analysis of whole-genome and transcriptome sequence data for identification of treatment options for metastatic triple negative breast cancer

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Triple negative breast cancer (TNBC) is characterized by the absence of expression of estrogen receptor, progesterone receptor and Her2-neu, accounting for 15% of all breast cancer diagnosis. Targeted therapies have a reasonable likelihood of improving the cure rate of early stage TNBC, and promising therapies discovered in the metastatic setting are rapidly advancing through clinical trials. We present integrated analysis of matched normal and tumor whole genome data, along with tumor transcriptome sequencing data of multiple individuals with metastatic chemo-resistant TNBC.

In each case, two independent 1.5kb Mate-pair libraries were generated for both tumor and germ-line derived genomic DNA and sequenced using SOLiD version 4.0 paired 50mers to a target of 30x depth. The tumor transcriptome was sequenced on four replicates and compared to transcriptome sequencing from ethnicity-matched population-based control hyperplastic breast tissue. Genome analysis was performed using multiple aligners and variant callers. Transcriptome alignment was performed using Life Technologies Bioscope pipeline, and differential expression analysis was performed using EdgeR and DESeq. We prioritized annotated germline and somatic variants by integrative analysis with differential expression results. Several striking examples of intronic events correlating with either altered splicing or differential expression were observed in genes relevant to cancer treatment, suggesting that transcriptomic data may have high value in interpreting somatic events that fall outside of coding regions. Final integration of data was validated through knowledge mining and convergence of somatic events and expression.

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P11.022

Using high-throughput SNP data to define the relationship between self-reported ethnicity and genetic identity in three Australian breast cancer studies

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Background.- Choosing the right subjects for a genome-wide association study implies, among other things, obtaining a sample with little or ideally no population structure. Even though efficient methods correcting for stratification now exist, these may fail when cases and controls come from entirely different populations. Subject recruitment is often based only on self-reported information about ethnic origin, but how well does this strategy deal with population structure issues?

Aim.- Here, we evaluate self-reported ethnicity as a predictor of genetic identity with respect to more objective information, such as patient/ parent/ grandparent place of birth in three Australian breast cancer studies.

Methods.- A total of 412 subjects from the (i) Australian Breast Cancer Family Study; (ii) Multiple Case Breast Cancer Families; and (iii) Australian Mammographic Density of Twins and Sisters were considered. Genotypes for 552,131 SNPs were determined with the Infinium® HD Human610-Quad BeadChip. Major population clusters were defined by means of principal component analysis (PCA) using EIGENSOFT. A naïve Bayes (NB) classifier was developed using self-reported ethnicity and place of birth as attributes and PCA clusters as the target value. Accuracy measures were used for the evaluation of the classification.

Results.- The sample showed a considerable level of stratification. PCA also revealed several inconsistencies, mostly concerning self-reported ethnicity, whereas the NB classifier indicated grandparent place of birth as the best predictor of genetic identity.

Conclusion.- Self-reported ethnicity, as opposed to grandparent place of birth, may not be the best guide for subject recruitment in epidemiological studies.

P11.023
Loss-of-Function Exomic Analysis in the identification of predisposition genes

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Exome sequencing has proved to be a powerful approach in the identification of causative variants underlying several Mendelian disorders. Two of the challenges that currently limit the widespread application of exome sequencing in disease gene identification are 1) the requirement for extensive, experienced statistical and bioinformatic infrastructure for sequence analysis and 2) the identification of insertion-deletion variants in short read data which impedes the ability to detect one of the commonest types of pathogenic mutation in human disease. We have been developing strategies to address these challenges. To address the first challenge we have been utilising and optimising a commercially available software, known as NextGENe, which uses a Burrows-Wheeler alignment algorithm to align short read data. We have analysed over 75 exomes from individuals with a variety of genetic conditions and to address the second challenge we have optimised indel calling and we have undertaken validation experiments. These showed that 48/54 (89%) insertion-deletion variants chosen randomly across our sample series were real. We have also developed a loss-of-function (LOF) script which identifies all variants predicted to result in premature protein truncation and have applied the script to all of the exomes. This has led to the identification of six truncating mutations that are pathogenic and several others that we are currently investigating. Our scripts allow the accurate detection and prioritisation of variants in exome sequencing that are most likely to be pathogenic and may be of value to other groups.

P11.024
A breast-cancer DNA methylation signature associated with field defect

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DNA methylation patterns have been recognised as cancer-specific markers with potential clinical application. We aimed at identifying differential methylation patterns between breast cancers and other breast lesions to establish a DNA methylation signature for breast cancer diagnosis. 117 fresh-frozen breast specimens including cancer, benign, normal-appearing breast tissue from patients and healthy individuals were analysed. Candidate genomic loci were isolated from DNA following bisulfite treatment and were hybridised to specialised oligonucleotide microarrays. A DNA methylation signature was identified by unsupervised analysis on the screening set, and its performance was assessed through bisulfite pyrosequencing in an independent validation set. The DNA methylation signature discriminates breast cancers from other samples in the screening set (10-12< adjusted p<10-6) (n=52) and is validated against an independent set of breast tissue samples (adjusted p<1.92e-07) (n=65). High sensitivity and specificity of the signature to detect breast cancer is demonstrated by Receiver Operating Characteristic (ROC) curves and the corresponding area under the curve (AUC) analysis (AUC> 0.90). This signature is constituted of 12 CpG sites associated with SFRP2 and GHSR genes, and show significant hypermethylation in cancers. Strikingly, the normal-appearing breast tissues from cancer patients are also methylated in these loci but at lower extent. This study reports a highly sensitive and specific molecular classifier for breast cancer detection based on DNA methylation. Furthermore, detection of methylated DNA in the normal-appearing breast tissues of cancer patients indicates an epigenetic field defect.

P11.025
Copy number variation and aberrations derived from cancer targeted exome resequencing

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Next generation DNA sequencing is rapidly becoming the platform of choice for numerous genomic applications. Complete genome sequencing is used to derive germline copy number variation (CNVs) and cancer copy number aberrations (CNAs) but its genomic resolution is limited by the extent of sequencing. Targeted resequencing (sequence capture) provides an option to increase the resolution and sensitivity of CNV and CNA analysis in a highly accurate and cost effective manner. For cancer genome analysis, targeted resequencing substantially increased the fold coverage over regions of interest, which is useful in more sensitive applications like CNA discrimination in complex tumor-normal tissue mixtures.

We have developed an experimental and statistical algorithmic solution to derive copy number variation from targeted resequencing on the scale of gene subsets up to whole exomes. We focused on four cancer samples with matched normal-tumor pairs including breast adenocarcinoma, colorectal carcinoma and lymphoma. To minimize experimental variance from the sequencing process, we used a novel indexing approach where matched tumor-normal pairs are sequenced together in a single sequencing lane. We enriched either gene subsets or whole exomes from these cancer samples. We then developed a statistical approach that incorporates absolute depth of coverage to provide a confidence in the tumor-normal read ratio. Using these combined procedures, we not only were able to confirm CNVs on the resolution of exons but also could discriminate indels smaller than 150 bases. Overall, our approach for CNV and CNA analysis on exome and exon subsets is robust and straightforward to implement.

P11.026***
Identification of low prevalence somatic mutations in heterogenous tumor samples

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To understand the progression and prognosis of cancer, researchers face a number of unique challenges. Current whole-genome sequencing studies are unable to cost-effectively achieve the required depth of coverage to discover rare mutations that represent 1% of a heterogeneous sample. Researchers have also been unable to routinely apply next-generation sequencing (NGS) technology to Formalin-Fixed Paraffin-Embedded (FFPE) samples, which are the standard archival method for nearly all solid tumors, due to challenges with low yield, degraded DNA, and sample extraction methodologies.

The RainDance DeepSeq™ FFPE Solution is the first ultra-deep targeted sequencing system for fresh-frozen and FFPE samples. Researchers can now interrogate as many as 500 targets across extensive collections of well-annotated clinical samples to discover rare cancer and other disease-specific mutations that represent as little as 1% of a heterogeneous sample.

To test the performance of the DeepSeq FFPE Solution, two matched breast and colon tumor samples from Fresh-Frozen Normal Adjacent, Fresh Frozen (FF), and FFPE Tissue were validated against a primer library targeting known cancer mutation hotspots across 46 genes. All samples were processed with the RainDance 2-Step Tailed Primer Assay and sequenced using the Illumina GAII with 100 base-pair reads. The data was analyzed and evaluated for sequence coverage and uniformity, along with the SNP detection and concordance between the FF and the FFPE tumor samples. Results demonstrate the ability for the RainDance DeepSeq FFPE solution to detect low prevalence mutations present in the FF and FFPE samples in both types of tissue.

P11.027

Expression profiling of rectal tumors defines genetic determinants of response to treatment

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BACKGROUND. To date, none of the identified signatures or molecular markers related to response to pre-operative chemoradiation (CRT) in locally advanced rectal cancer (LARC) has been successfully validated as a diagnostic or prognostic tool applicable to routine clinical practice. We applied DNA microarrays to gain insight into the molecular signatures associated with response to treatment after CRT.
METHODS. RNAs from patients with LARC without metastasis were obtained and subsequently cDNA and cRNA were synthesised for hybridization on *Human WG CodeLink bioarrays*. Tumor tissue biopsies were obtained before CRT. Significant genes were found using *Significance Analysis of Microarrays*. Real time quantitative RT-PCR (qRT-PCR) was used to confirm the results from the arrays. Results were correlated with pathological response (*Mansard's* criteria).
RESULTS. We used a t-test to find those genes that have an expression significantly different between responders and non-responders patients. We found 257 genes with an adjusted p<0.05, which clearly differentiate these two groups of patients after CRT. A detailed analysis identified genes encoding proteins associated with several canonical pathways, such as Pyrimidine and Purine Metabolism, and Colorectal Cancer Metastasis Signaling. As occurred in the microarrays, the level of expression of MYC determined by qRT-PCR, showed significant differences between Responder and Non-Responder subgroups.
CONCLUSIONS: Molecular signatures could provide the basis for improved treatment stratification of patients with LARC and define novel prediction of response to CRT treatment. Elevated levels of MYC not only induce growth and proliferation but also strongly could sensitize tumor cells toward pro-apoptotic stimuli including DNA damaging agents.
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P11.028

Identification of post-transcriptional regulatory elements in CDK5R1 3'UTR gene involved in CNS development and functioning

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CDK5R1 encodes p35, an activator of CDK5, which is involved in neuronal migration and differentiation during CNS development. It has been implicated in some neurodegenerative disorders and candidate for the non-specific mental retardation. The considerable size of CDK5R1 3'UTR and the high level of sequence conservation suggests a role in post-transcriptional regulation. We recently reported that the large CDK5R1 3'UTR contains some regulatory elements affecting transcript stability. Precisely, a 138 bp region was shown to be the most destabilizing region within the 3'UTR. We search for post-transcriptional destabilizing factors in this region by UV-cross-linking that showed the binding of some RBPs among which the strongest band had a molecular weight compatible with that of the nELAV confirmed by immunoprecipitation. The fragment has been further divided in four regions for UV-crosslinking experiment that allowed to identify a stretch of 7 bp binding region and through the site-directed mutagenesis we identified the binding sequence. Silencing and overexpression of this factor are in progress in order to validate its destabilizing activity on CDK5R1 expression. To verify the binding of further regulatory elements the four regions of the 138 bp fragment have been transcribed and used for pulldown experiment of protein extracts from SK-N-BE cells. Following mass spectrometry analysis we identified the hnRNPA2/B1 for which the cytoplasmatic function is unknown and should be confirmed by immunoprecipitation assays. This study, besides defining the regulatory mechanisms of CDK5R1 expression, may help to identifying the pathogenetic implications of the gene in neurodegenerative and cognitive diseases.

P11.029

Methylation status of the cyclin-dependent kinase 2A (p16INK4a/CDKN2A) promoter in human atherosclerotic plaques

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Genome-wide association studies have linked common single nucleotide polymorphisms on chromosome 9p21.3 with risk of atherosclerotic diseases; however the underlying mechanism remains unknown. This genomic region contains several genes including the *CDKN2B* (p15INK4b) and *CDKN2A* (p16INK4a and p14ARF), which are key regulators of the cell cycle. Epigenetic inactivation of the *CDKN2A* gene by CpG islands methylation is well known in cancerogenesis and aging. The aim of the present research was to determine whether such methylation occurs in the atherosclerotic disease. Methylation-sensitive PCR using Hpa II restriction was employed in studying of the p16INK4a/CDKN2A promoter methylation in tissue samples of carotid artery (54 atherosclerotic tissues and 54 paired macroscopically normal specimens from the same patients). The promoter was not methylated in all studied tissue samples. This research was supported by Grant of Russian Foundation of Basic Research (10-04-00674).

P11.030

Microarray-based DNA methylation analysis of human atherosclerotic plaques

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The contribution of epigenetic mechanisms to cardiovascular diseases remains poorly understood. Hypomethylation of genomic DNA is present in human atherosclerotic lesions and methylation changes also detect at the promoter level of several genes involved in the atherogenesis. In the current study for the first time quantitative microarray-based methylation profiling of 1505 CpG-sites across 807 genes was performed in two samples of atherosclerotic aorta and carotid artery wall lesions using the GoldenGate Methylation Cancer Panel I (Illumina). We identified 103 CpG sites (associated with 90 genes) that varied significantly in the DNA methylation level between the tissue samples. The most pronounced differences in the DNA methylation level were registered for a site which is located -541 bp from the transcription start site of imprinted gene *H19*. In particular, promoter's site showed a low level of methylation in the sample from the aorta's plaque area (0.02) compared to carotid artery (0.66). By comparing 90 genes that were differentially methylated between tissue samples in our study, 10 genes (*ICAM1*, *GSTM1*, *IGFBP1*, *POMC*, *APOA1*, *IL1RN*, *INS*, *LTA*, *MMP3*, *THBS2*) were overlapped with data from Human Genome Epidemiology Network (HuGENet), in which they were identified as candidates for cardiovascular disease continuum. This study was supported by state contract of the Ministry of Education and Science (Russia) for Science and Educational Centers (N 02.740.11.0281).

P11.031

Haplotype analysis of MCP-1/CCL2 with essential hypertension in tatars from Russia

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Objective: Inflammation play an important role in the initiation essential hypertension (EH). Evidence suggests that the chemokine, monocyte chemoattractant protein-1 (MCP-1; gene name, CCL2, previously SCYA2) is involved in EH. In the present study, we examined a possible association between polymorphic variants -5796A>T(rs1860190),

-2581A>G(rs1024611), 704(14)I/D (rs3917887) and 5357C>T (rs991804) of the CCL2 gene and EH.
Subjects and methods: We analyzed the association of genetic variation in the CCL2 gene in groups of hypertensive patients and healthy subjects from Tatar ethnic group (Bashkortostan, Russia). MCP-1 genotypes were determined in 164 and 209 individuals, respectively. DNA was isolated from whole venous blood using phenol-chloroform extraction by standard method. Genotypic frequencies in control subjects for each SNP were tested for departure from Hardy-Weinberg equilibrium using a Fisher`s exact test. Haplotype block structure was determined with Haploview 4.2 program using EM algorithm.
Results: In this study, we have successfully genotyped four SNPs of the CCL2 gene in 373 individuals. To test the association between MCP-1 and risk of EH, we determined 12 common haplotypes , that accounted for 75% of all haplotypes. The EH patient group showed a significant higher frequency of the CCL2*A*G*D*T haplotype compared to the controls (20.5% versus 13.5%, respectively; χ2=6.662, P=0.0099 Pperm=0.0472, OR=1.53). Thus, CCL2*A*G*D*T haplotype has association with EH.
Conclusion: Our data demonstrate that MCP-1 is significantly associated with the risk of occurrence EH, but the certain mechanism of this influence remains to be elucidated.

P11.032
A study of the Insertion-Deletion polymorphism of the gene β-2 receptor of bradykinin (BDKRB2)

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INTRODUCTION. Described in the literature that polymorphic variants of genes encoding components of the kallikrein-kinin system are associated with physical performance rights. The presence of favorable alleles of this system provides a more effective implementation of physical work and, therefore, contributes to better results in various sports. The study aims to estimate the frequency distribution of genotypes and alleles of gene ß2-bradykinin receptor (BDKRB2) in individuals with different coefficients of endurance. MATERIALS AND METHODS. We studied samples DNA of 199 of individuals with different values of endurance aged 18-30 years. Endurance coefficient (EC) is determined to assess the state of the cardiovascular system in accordance with formula Kvaasa: EC=heart rate×10/pulse pressure. The analysis genetic polymorphism is realized by polymerase chain reaction (PCR). Statistical data processing was carried out using a software package ANOVA (spss v13). RESULTS. Analysis of the frequencies of genotypes and alleles of gene ß2-bradykinin receptor (BDKRB2) between groups with different endurance factor revealed a significant increase in the frequency of the genotype *D/*D and allele *D in the group with increased activity of the cardiovascular system (P=0.04,x2=3.951). The data obtained are of interest for understanding the molecular and genetic mechanisms of predisposition to perform physical activity. This work was partially funded by the grant of Russian Foundation for Humanities 10-06-84609.

P11.033
Morpholino knockdown of the zebrafish CENTA2 orthologue results in cardiovascular defects

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Cardiovascular malformations (CVMs) have a higher incidence in patients with NF1 microdeletion syndrome, compared to classical NF1 patients (18% vs 2%), presumably owing to haploinsufficiency of genes lying in the deletion interval and important for cardiac morphogenesis. One of the possible candidate genes for CVMs onset inside the deletion, CENTA2, was found, by WISH (Whole-mount In Situ Hybridization) on mouse embryos, to be turned on in heart at 9-9.5 dpc, when the heart tube is looping and the valves and septa are forming, suggesting a role in heart development. In order to provide further evidence on a possible role of CENTA2 in cardiac development, we employed zebrafish as a model system. Zebrafish is a useful model that allows a detailed cardio-vascular analysis even in embryos with severe defects that would be lethal in other organisms. We performed loss-of-function experiments injecting a morpholino that is able to block the mRNA translation of the zebrafish CENTA2 orthologue, *centa2-like*. At 2 dpf the injected embryos displayed *in vivo* circulatory and cardiac defects, such as blood stases in the head and caudal region, block of circulation and heart shape defects. A preliminary molecular characterization on morphants at 2 dpf showed that the injection of the *centa2-like* morpholino caused heart looping defects, in particular the formation of a tubular heart or an inverted heart looping. WISH analysis on morpholino-injected embryos at different developmental stages with different cardiac markers will allow further investigation of the role of *centa2-like* during zebrafish cardiac morphogenesis.

P11.034
The first sequenced carnivore genome shows complex host-endogenous retrovirus relationships

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Host-retrovirus interactions influence the genomic landscape and have contributed substantially to mammalian genome evolution. The dog has emerged as an excellent model for complex disease genetics in human. Therefore, we analyzed a female boxer (Canis familiaris) genome for complexity and integration pattern of canine endogenous retroviruses (CfERV) to gain further insights into comparative evolution regarding the human genome. Intriguingly, we could only identify 407 CfERVs constituting 0.15% of the dog genome, compared to six times more ERVs identified in the human genome. Primates and canines have likely different mechanisms to purge, restrict and protect their genomes against retroviruses. The CfERV integration landscape showed a non-uniform intra- and inter-chromosomal distribution in agreement with distinct selective pressures at different loci. Evidence for selection against CfERVs in sense orientation relative to chromosomal genes was supported by a majority of CfERVs integrated in antisense orientation within 100 kb from annotated protein-coding genes. Finally, a novel group of gammaretrovirus-like CfERVs with high similarity to HERV-Fc1 was found to have potential for active retrotransposition and possibly result from lateral transmissions between dog and human as a consequence of at least 10.000 years of close interactions. In conclusion, this ERV analysis of the first sequenced carnivorous species supports the notion that different mammals interact distinctively with endogenous retroviruses and suggests that retroviral lateral transmissions have occurred between dogs and. This comprehensive CfERV study will provide an excellent resource for dog-to-human comparative genomics.

P11.035
Impact of cis-regulatory motifs in non-coding regions on CFTR expression

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Cystic Fibrosis is a rare and lethal autosomal recessive disease. Although *CFTR* shows all the features of a housekeeping gene, it is tightly regulated both spatially and temporally. In addition to several regulatory motifs identified within the minimal promoter, (Pittman et al. 1995, Taulan et al. 2007, René et al. 2010, Lopez et al. 2010), this gene is also post-transcriptionally regulated by ARE (AU-rich elements) motifs in its 3'UTR region (Baudoin-Legros et al. 2005).
However, tissue- and developmental-specific regulatory elements governing *CFTR* gene expression are still not defined. The aim of this work is to identify the most of functional motifs involved in the basal *CFTR* gene expression control.
Use of bioinformatic tools leads to the identification of new putative regulatory sites, binding sites for transcription factors in 5'UTR (TFsearch, consite...), and for specific proteins (AREsite) and

microRNA (TargetScan, miRBase...) in non-coding 3'UTR part.
To assess impact of each motif, we have carried out many constructs, countaining or not, natural or experimental mutants. Reporter gene assays, quantification of *CFTR* transcript level, RNA stability studies..., were performed to test functional significance of each mutation.
Here, we have identified new binding sites for essential transcription factors in the promoting part (as C/EBP proteins, another motif as previously described by Pittman et al. 1995). We have also shown the role of some microRNA (as hsa-miR-101) on the *CFTR* gene expression regulation. These *cis*-regulatory motifs might constitute new therapeutic stategies to modulate transcriptional and post-transcriptional regulation of *CFTR* gene.

P11.036
Integrative analysis of gene coexpression networks identifies novel ciliary proteins in human tissues

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Cilia are multifunctional cell organelles whose dysfunctions cause ciliopathies - an emerging group of human genetic diseases. Understanding molecular biology of cilia requires genome-wide identification of proteins involved in biogenesis and function of this organelle. Here, we explored the human ciliome by constructing gene coexpression networks in 10 public microarray datasets from 3 tissues containing cells with motile cilia (airways, fallopian tubes and brain). The network analysis identified a cilia-related module of coordinately expressed genes shared by the tissues. This transcriptomic signature recapitulated 294 known ciliary genes (including ciliopathy genes DNAH11, DPCD, HYDIN, LCA5, MDM1, RSPH4A, RSPH9) and identified 77 novel genes with expression patterns specific to ciliated cells. We used data from the Protein Atlas project to validate their expression at the protein level: for 138 proteins we found an increased staining in the ciliary subcellular compartment or the apical region of the cytoplasm. We finally searched for genes with differences in expression between the ciliated tissues and found SLC47A2 as a gene highly expressed in ciliated cells in brain only. SLC47A2 represents one of the few known examples of differences between ciliated cells across tissues. This protein belongs to multidrug and toxin extrusion transporters (MATE) and may play a role in efflux of metabolites and xenobiotics from brain. Our analysis provides novel insights into the human ciliome and illustrates how combinations of open-access transcriptomic and proteomic resources can be utilized to annotate protein function at a genome-wide scale. This work is partly supported by grant RFBR 10-04-01385-a.

P11.037
Copy number variation and chromatin structure

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The functional consequences of structural variation in the human genome range from adaptation, to phenotypic variation, to predisposition to diseases. We, and others, have previously shown that alteration in copy number of a given genomic region influences the expression of genes mapping within, but also on its flanks. To assess the possible mechanism(s) behind this neighboring effect, we gauged the histone modification status by ChIP-seq in human lymphoblastoid cell lines from patients affected by Williams-Beuren, Williams-Beuren region duplication, Smith-Magenis or DiGeorge Syndrome and control individuals. We monitored monomethylation of histone H4 K20 and trimethylation of histone H3 K27, as proxies for open and condensed chromatin, respectively.
Sequences were mapped using Bowtie, while SICER and DESeq were used to define regions with modified histones. Upon comparison between samples we observed that the rearranged regions (22q11.2, 17p11.2 and 7q11.23) were significantly modified in their chromatin structure. Consistent with the changes in expression levels of multiple genes mapping on the entire length of the chromosomes affected by structural variants, we also detected regions with modified histone status between samples, up- and downstream from the critical regions, up to the end of the rearranged chromosome. Coherently, we pinpointed alteration of chromosomal looping interactions between affected gene loci and the rearranged interval using an unbiased variant of chromosome conformation capture (3C-seq).
We conclude that large genomic rearrangements can lead to changes in the state of the chromatin spreading far away from the critical region, thus possibly affecting expression globally.

P11.038
A practical method for high-resolution CNV estimation and validation using digital PCR

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Digital PCR (dPCR) is a practical solution for validating copy number variations identified by next generation sequencers and microarrays. The dPCR method we developed empowers one person to screen hundreds of samples for CNV analysis in a single work shift. The dPCR workflow involves using restriction enzymes to separate tandem copies of a target gene prior to assembling a duplex TaqMan assay that includes reagents to detect both the target gene and a single-copy reference gene. The reaction mixture is then partitioned into 20,000 nanoliter droplets that are thermo-cycled to end-point before being analyzed in a two-color reader. The fraction of positive-counted droplets enables the absolute concentrations for the target and reference genes to be measured, from which, a relative copy number is determined. 20,000 PCR replicates per well provide the statistical power to resolve higher-order copy number differences. This low-cost method reliably generates copy number measurements that cluster near integer values without overlap with adjacent copy number states allowing consistent discrimination between four and five copies at 95% confidence level. Lastly, to our knowledge, this technology is the first to be capable of phasing copy number variants, as we can easily determine whether all copies of a target locus are on the same or different chromosomes. Applications of this technology include: validation of CNV discoveries from GWAS, follow-ups to next generation sequencing experiments, cytogenetic analyses, copy number alterations in cancer, and CNV phasing.

P11.039
The Effect of Sulforaphane on Transcriptional Repression and Gene Expression of CoREST

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Sulforaphone (SFN), a compound derived from cruciferous vegetables like broccoli, is reported to inhibit HDAC activity in prostate cancer cells. Aberrant activity of HDACs has been documented in several types of cancers and HDAC inhibitors (HDACi) have been employed for therapeutic purposes. CoREST, a subunit of HDAC1, HDAC2 and LSD1, plays a role in blocking the expression of key target genes. In this study, we aimed to investigate the effect of sulforaphane on the transcriptional repression of CoREST in prostate cancer cell lines PC3 and V-CAP, by chromatin immunopresipitation method (ChIP) and gene expression analysis. The cytotoxicity (IC50) of sulforaphane was found as 31.95 and 27.5 µg/ml in V-CAP and PC3 respectively, by XTT method. The CoREST ChIP relative ratios in control and IC50 dose groups were 19.08 and 9.87 in PC-3, while it was 17.37 and 6.28 in V-CAP cell lines. The gene expression analysis of CoREST in control and IC50 dose group was found as 0.0036 and 0.0028 in PC-3 cell line and 0.00026 and 0.00022 in V-CAP cell line. The transcriptional repression of CoREST was decreased by 51.74% and 36.16% and gene expression was decreased by 79.3% and 84.90% in PC-3 and V-CAP cell lines.
The HDAC inhibitor effect of Sulforaphane may depend on the decrease of the CoREST gene expression in PC-3 and V-CAP cells, and additional studies with a wide range of cell lines are needed to evaluate this compound as a new approach for the treatment of prostate cancer.

P11.040

Molecular Profiling of Human Sporadic Craniosynostosis

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Craniosynostoses (CRS), the premature fusion of cranial sutures, are the more prevalent human craniofacial malformation, often associated to brain abnormalities, mental retardation and relevant morbidity. The molecular pathogenesis of CRS is still largely unknown. This study attempted to clarify the molecular mechanisms underlying the premature ossification of calvarial sutures. Ten sporadic non-syndromic CRS patients were enrolled in the study upon obtaining informed consent and excluding known mutations in the FGFRs and TWIST genes. Calvarial cells (CC) were isolated from prematurely fused and normal unfused suture surgical specimens. CC displayed a homogenous mesenchymal IP (CD29+,CD44 +,CD105 +,CD73 +,CD34-. CC morphological features and in vitro osteogenic potential, along with the expression of osteospecific genes was analyzed comparatively in fused and unfused suture-samples. CC isolated from pathological sutures showed a premature osteogenic commitment along with the augmented expression of upstream osteogenic genes (LMP, RUNX2, NELL-1 and OC). Moreover, genome-wide expression profiling was performed at the exon level using microarray analysis on total RNA isolated from matched suture specimens of each patient. Up-regulated genes included ADAM21, involved in cell-cell/cell-matrix interactions during tissue development; CLCF1, a potent neurotrophic factor implicated in structural head and spine abnormalities. Interestingly, FGFR1, FGFR2, FGFR3 and TWIST1 were among significantly alternatively spliced genes. This suggested that possible post-transcriptional regulation of gene expression occurring at the somatic level could be one possible pathogenic mechanism in sporadic CRS. The identification of genes, whose function is impaired at the somatic level in CRS, would help the future development of targeted molecular therapy.

P11.041

Identification of cRSS with close structural similarity to RSS of Ig and TCR genes in the human genome

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Cryptic recombination signal sequences (cRSS) are motives that potentially can mediate instability of mammalian genome, when V(D)J recombination system gets out of control. It is known that there are a lot of cRSS in the human genome outside the Ig and TCR loci. However, at present no information is available about origin, quantity and genomic location of cRSS with close structural similarity to RSS of functional V, D, J segments of Ig and TCR genes (fRSS).

Using specially developed program algorithms we found that there are 580 cRSS in the human genome (Build 37.1), which have close structural similarity to fRSS. Only 67 of these cRSS were found in duplicated V, D segments of Ig and TCR genes. Appearance of 32 % cRSS in the genome may be explained by random nucleotide combinations. We observed that 27, 14, 3, 3 and about 1 % cRSS are structural elements of non-LTR retrotransposons (AluJb, L1MDa, etc.), endogenous retroviruses and LTR retrotransposons (ERVL-E-int, MER65B, etc.), simple repeats ((CA)n, (CAGA)n, etc.), DNA transposons (Charlie25, MER5B, etc.) and other repeats respectively. In 85 % cases the nucleotide sequences of motives are fully localized inside repeats. 195 (34 %) cRSS were found in 189 (0,8 %) protein-coding genes (GRIP1, PDE8A, etc.).

Our study shows that cRSS with close structural similarity to fRSS can be found in many human protein-coding genes. Their appearance in the human genome can be explained by duplications of Ig and TCR gene fragments, spread of repeats and random nucleotide combinations.

P11.042

Epigenetic regulation of the X chromosomal macrosatellite repeat encoding for the cancer testis gene CT47

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Macrosatellite repeats (MSR) present an extreme example of copy number variation, yet their epigenetic regulation is largely understudied. The CT47 cancer-testis antigen array on human Xq24 consists of 4.8kb-large units and the CT47 gene encoded in each unit is expressed in testis and in certain types of cancer, but not in non-malignant somatic tissue. We studied CT47 for possible correlations between copy number variation, epigenetic regulation and transcriptional activity in normal and malignant cells. In lymphoblastoid cell lines and in primary fibroblasts CT47 transcripts were absent, corroborating the observed heterochromatic structure and DNA hypermethylation of the CT47 promoter. Heterochromatinization of CT47 occurs early in human embryonic stem cells showing high levels of DNA methylation and repressive chromatin modifications in the absence of CT47 expression. In small cell lung carcinoma cell lines showing low levels of CT47 transcripts we observed reduced levels of H3K9me3 and H3K27me3 without increase in euchromatic histone modifications. DNA methylation levels in the CT47 promoter region are also significantly reduced in these cells. Apparently during oncogenic transformation there is a relative loss of repressive markers resulting in leaky expression of CT47. Comparing the epigenetic regulation of CT47 with other published MSR like the autosomal MSRs TAF11-Like, PRR20, ZAV and D4Z4 which is involved in facioscapulohumeral muscular dystrophy, a model emerges in which some MSR seem to be governed by common regulatory mechanisms with their abundant expression mostly being restricted to the germ line.

P11.043

Automatization of CYP2D6 genotyping in patients under paroxetine treatment

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Cytochrome P450 (CYP) 2D6 is one of the most investigated from CYP family in relation to genetic polymorphism. There is a wide interindividual variability in the enzyme activity of CYP2D6. Typical substrates for CYP2D6 are largely lipophilic bases and include antidepressants, antipsychotics, antiarrhythmics, antiemetics, ß-blockers and opioids. The CYP2D6 activity vary within a population and includes ultrarapid metabolizers (UMs), extensive metabolizers (EMs), intermediate metabolizers (IMs) and poor metabolizers (PMs). There is a considerable variability in the *CYP2D6* alelles distribution among different ethnic groups, resulting in variable percentages of PMs, IMs, EMs and UMs in a given population.

In our study psychiatric patients under the paroxetine therapy have been automatically genotyped by using INFINITI Analyzer (Autogenomics Inc). The INFINITI™ Analyzer is an automated, multiplexing, continuous flow, random access microarray platform that integrates all the discrete test processes such as sample handling, reagent management, hybridization, stringency and detection for analyses of DNA into totally self-contained system. It can be used for clinical multiplex systems intended to measure and sort multiple signals from clinical sample. INFINITI analyzer is designed to measure fluorescence signals of labeled DNA target hybridized to BioFilmChip™ microarrays. It automates assay and integrates all the discrete processes of sample handling, reagent management, hybridization, detection, and results analysis. Assays are processed automatically and read by the built-in confocal microscope. Results are analyzed and presented in numerical and graphical format. The genotype has been determined and compared with clinical data, phenotype, drug dosages and compliance. The results are described in included tables.

P11.044
Assessing DNA copy numbers in large-scale studies using genomic arrays

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Oligonucleotide-based arrays are commonly used to assess genotype-phenotype association on a genomic scale, and studies involving DNA copy numbers are becoming more common. While genotyping algorithms are largely concordant when for example assessed on HapMap samples, methods to assess DNA copy number alterations often yield discordant results. One explanation for the discordance is that DNA copy number estimates are particularly susceptible to systematic differences that arise across batches of samples that were processed at different times or by different labs. Analysis algorithms that do not account for such systematic biases are more prone to spurious findings that will not replicate in other studies. This presentation describes statistical methods and software for the locus-level estimation of DNA copy number that specifically adjust for batch effects. We illustrate a workflow for copy number analysis using HapMap Phase 3 data, including normalization, genotyping, adjusting for batch effects in copy number estimation, visualizations to inform downstream processing, and smoothing point estimates as a function of physical position via segmentation and hidden Markov models. We also discuss the importance of GC content, and show a case study on inferred and validated (via RT-PCR) de-novo deletions in trios with cleft-palate probands. We also provide a performance assessment of four different copy number microarray platforms using a spike-in experiment.

P11.045
ICF syndrome, a model to investigate how DNA hypomethylation affects DNA replication

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In addition to regulating gene expression, epigenetic modifications contribute to the control of other biological processes, such as DNA replication, but their relationships is not well understood. Using cells from ICF (Immunodeficiency, Centromeric instability and Facial anomalies) patients, we investigated how a DNA methylation defect affects DNA replication in human cells. ICF is a rare autosomal recessive disorder resulting from impairment of the DNMT3B protein, a *de novo* DNA methylating enzyme. ICF patients suffer from a severe immunodeficiency and are characterized by loss of DNA methylation in repetitive sequences and decondensation of constitutive heterochromatin and chromosome instability. Previously, we showed that heterochromatic genes undergo hypomethylation and escape silencing in ICF cells. DNA hypomethylation was generalized to all the heterochromatic genes and to all the ICF cell lines, whereas gene expression was restricted to some genes, every patient having his own group of activated genes. Herein, we showed that heterochromatic genes replicate earlier in the S phase in ICF compared to control cell lines. The change in the replication timing was not correlated with gene activation. Next, to investigate further the effect of DNA hypomethylation on DNA replication, we measured fork speed by molecular combing and S-phase length by flow cytometry. Replication fork speed was higher and the S-phase was shorter in ICF cells suggesting that the alteration of DNA replication is not restricted to heterochromatin but is generalized to the whole genome. ICF cells are an ideal model to investigate DNA methylation changes and their molecular and pathological consequences.

P11.046
Analysis of DNA Lesions Induced by Ultraviolet Radiation with Two-Dimensional Electrophoresis

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Background. The main DNA damage caused by ultraviolet radiation (UV) are base changes that cause bending of DNA molecules. They include cyclobutane pyrimidine dimers, pyrimidine (6-4) pyrimidone photoproducts and its Dewar isomers. Two-dimensional electrophoresis, a method that can separate bent DNA from normal DNA, was used to analyse DNA lesions in complex DNA samples and cell cultures after UV radiation.

Methods. Human genomic DNA was digested with MboI and radiated with UVB (312 nm, 5-30 J/cm2) in a droplet on a Petri dish. HeLa cell culture was radiated with UVB (15-45 J/cm2), the DNA isolated and digested with MboI. The samples were analysed using Two-Dimensional Strandness-Dependent Electrophoresis (2D-SDE) and Two-Dimensional Conformation-Dependent Electrophoresis (2D-CDE). AvaI digested lambda DNA was UVA radiated (44 J/cm2) and analyzed in the same manner.

Results. DNA induced by UVB migrated in front of normal DNA on 2D-CDE gel, due to bending caused by the DNA lesions. UVA radiation of lambda DNA also caused migration of DNA in front of normal DNA with 2D-SDE. Surprisingly, 2D-SDE analysis of DNA radiated with UVB revealed formation of a DNA arc behind normal DNA. The ratio of this arc increased with increased UVB dosage up to 43%. The same effect was detected in DNA from UVB induced cell culture.

Conclusions. 2D-SDE and 2D-CDE can be used to assess DNA lesions associated with UV-radiation. The DNA arc caused by UVB radiation may be explained by cross-links or formation of A-helix DNA due to either base lesions or deoxyribose oxidation to ribose.

P11.047
LUPA: Discovering human disease susceptibility loci via the power of dog genetics

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Given the many human diseases that exert increasing social and economic burdens on society, there is a compelling need to propose novel therapeutic strategies. As innovative therapies are best designed based on the underlying genetic and molecular mechanisms of the disease, state-of-the-art research must be conducted on animal models of human pathologies. The domestic dog is an exemplary genetic resource for the dissection of human disease pathways. Due to intensive selection for desirable traits in a pet animal, each dog breed is characterized by a highly homogenous genetic structure. A spectrum of equivalent diseases afflicts both man and dog and exhibits very similar pathological symptoms. The EU-funded project LUPA has conducted extensive genome-wide association studies on a collection of dog cohorts diagnosed with a range of disease conditions including cancer, cardiovascular, inflammatory, neurological and monogenic diseases. Given its collaborative nature, LUPA has leveraged on a network of veterinarians for diagnosis and sample collection, a centralized high-throughput genotyping facility, and geneticists and computational biologists for data analyses. Since 2008, with ~10,000 samples genotyped, a number of associated loci have been identified for various diseases, including a region on CFA 5 for cardiovascular, DLA class II alleles for immunological and *CCDC39* for respiratory disorders. LUPA has successfully built an infrastructure of resources, most notably a new SNP array and a comprehensive database of well-characterized phenotypes and SNP genotypes. The achievements of LUPA are key to facilitating investigations in comparative and translational studies in partnership with the human disease research community.

P11.048
High resolution map of canine copy number variation

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Copy number variations (CNVs) are an important form of structural variation in mammalian genomes. Substantial amount of phenotypic differences between healthy individuals have been attributed to CNVs and they also contribute to disease risk in several human disorders including autism spectrum disorders, schizophrenia and epilepsy. Dog breeds as genetic isolates have recently emerged as a new model for human inherited diseases. Hundreds of genetic diseases have been identified in dogs, many of which are similar to human diseases and are likely to have a similar genetic background. CNVs are expected to play a role in several disease conditions in dogs and contribute to breed-specific characters. This study aims to map and catalogue CNVs in 50 dogs from 17 breeds (2-10 dogs/breed) and 3 wolves by comparative genome hybridization (aCGH) using Nimblegen's arrays with 2.1 million probes giving a high resolution of approximately 1 kb median probe spacing across the canine genome. This density gives considerably higher resolution than previously published studies. A Finnish Boxer DNA was used as a reference for other breeds. The results of this high resolution analysis of the CNVs in a large number of breeds provide important discoveries likely to contribute to several phenotypes in dogs and are being followed up in many cohorts available in the European canine genomics effort, LUPA consortium. Furthermore, findings associated with genetic diseases can be tested on human cohorts and eventually this will improve the health of both species. The new CNV data will be discussed in the meeting.

P11.049
Evaluation of global DNA methylation and twelve polymorphisms of folate pathway on Down syndrome etilogy

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Down syndrome (DS) is, in most cases, attributed to maternal non-disjunction which occurrence is associated with DNA hypomethylation as consequence of abnormal folate metabolism. Thus, global methylation was compared between 10 mothers of individuals with DS and 10 of individuals without the syndrome. Association between DNA methylation, *Methylenetetrahydrofolate reductase (MTHFR)* C677T and A1298C, *Methionine synthase* (*MTR)* A2756G, *Reduced folate carrier 1 (RFC1)* A80G, *Transcobalamin 2 (TC2)* A67G and C776G, *Cystathionine beta-synthase (CβS)* 844ins68, *Methylenetetrahydrofolate dehydrogenase 1* (*MTHFD1)* G1958A, *Serine hydroxymethyltransferase* (*SHMT)* C1420T, *Methionine synthase reductase* (*MTRR)* A66G, *Betaine-homocysteine methyltransferase (BHMT)* G742A and *Dihydrofolate reductase* (*DHFR*) deletion of 19 base pair (bp) polymorphisms, serum folate and plasma homocysteine (Hcy) and methylmalonic acid (MMA) concentrations was also evaluated. Global methylation analysis was carried out by *Imprint Methylated DNA Quantification Kit* (*Sigma Aldrich*). The investigation of polymorphisms was performed by Polymerase Chain Reaction (PCR), Real time PCR and PCR followed by digestion. Plasma MMA and Hcy concentrations were determined by liquid chromatography-tandem mass spectrometry and folate by chemiluminescence. Percentage of methylation between DS mothers and non-DS mothers did not differ (P=0.19). *DHFR* 19 pb polymorphism was asscociated with global DNA methylation with a mean of DNA methylation percentage in heterozygous genotype mothers (ID) of 24.67 ± 6.51%, mothers DD 18.73 ± 5.65% and II, 14.32 ± 9.62% (P=0.05). This work is the first to measure global methylation in DS mothers and association between *DHFR* 19 bp polymorphism and DNA methylation shows the importance of abnormal folate metabolism on DS etiology.

P11.050
The transcription factor MEIS1 marks a megakaryocyte-specific alternative promoter of the DNM3 locus at the position of a GWAS sequence variant for platelet volume

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We recently identified 12 quantitative trait loci for mean platelet volume and three for platelets count by a Genome-Wide Association Study (GWAS). One of the association single nucleotide polymorphisms (SNPs), rs10914144, lies in intron 2 of the DNM3 gene on chromosome 1q24.3.
We set out to elucidate the mechanism by which sequence variation in the DNM3 locus exerts its effect on platelet volume.
First we reproduced the GWAS association in platelets from two groups of 19 healthy individuals each, who were homozygous for the major and minor alleles of the association SNP (P<0.05, unpaired t-test). We then measured DNM3 transcript abundance and observed significant differences between the groups. Chromatin immunoprecipitation (ChIP) with massive parallel sequencing for the MK-specific transcription factor MEIS1 in the MK-like cell line CHRF-288-11 identified four MEIS1 binding sites within the DNM3 locus. One site in intron 2 of DNM3 harbours a SNP (rs2038479) 10,600 bp downstream of the original association SNP, with which it is in strong linkage disequilibrium (r2=0.941). Sequencing of MK mRNA and 5' RACE identified a novel, alternative DNM3 transcript that lacks the first two exons and instead contains a novel exon, named 2B, which is located in intron 2. Real-time qPCR analysis demonstrated that in MKs transcript 2B is predominant whilst being virtually absent from neuronal cells. Promoter studies identified an alternative promoter upstream of exon 2B which is only active in MKs but not in neuronal cells. Studies are currently underway to assess DNM3 protein function in megakaryopoiesis and platelet formation.

P11.051
Using an EMT in vitro model to approach novel intronic based-mechanisms underlying pathological cadherin impairment

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Epithelial mesenchymal transition (EMT) is a fundamental mechanism controlling multiple events during embryonic development and cancer. The loss of E-cadherin expression, and consequently of cellular adhesion, is a key event in this phenomena. Cancerous cells undergoing EMT, exhibit a mesenchymal-like phenotype with concomitant polarity loss, increased invasibility and apoptosis resistance, enabling metastization. Many proteins have been proved to induce EMT and others to be modulated by EMT, but nothing has been explores in terms of intronic transcription modulation during this process.
To better understand the role of intronic-based transcription during EMT, we have reproduced this process in vitro by treating a normal mouse mammary cell line with TGF-β1. DNAse treated RNA extracted at distinct EMT-timepoints was then subjected to RNA-sequencing. We confirmed the occurrence of EMT via analysis of the differential transcription of epithelial and mesenchymal markers and the activation of the TGF-beta pathway underlying EMT. Moreover, we were able to identify both differential and constantly transcriptionally active intronic areas within cadherin genes, which putatively underlie novel cis-regulatory elements. In addition we have detected a differential rate of transcription from within introns and exons of both CDH1 and CDH2 genes, both hallmarks of epithelial and mesenchymal states of EMT. These observations point out a specific role for CDH1 and CDH2 intronic transcription in EMT.

P11.052
Epigenetic effects of prenatal exposure to ethanol: a specific effect on the H19 gene methylation in sperm

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Alcohol exposure during pregnancy, in humans and mice, induces a wide range of disorders in the offspring referred to as the fetal alcohol

syndrome (FAS). Epigenetic alterations like, in particular, methylation changes in some imprinted genes may play a role in the teratogenic effects of alcohol. The aim of our study was to evaluate the possible effects of alcohol administration in pregnant mice on the methylation pattern of 5 imprinted genes (H19, Gtl2, Peg1, Snrpn and Peg3) in the tail, liver, skeletal muscle, hippocampus and sperm DNAs of the male offspring over 2 generations. The effect observed on the imprinted genes was a small (3 %) but highly significant decrease in the number of methylated CpGs of H19, in the F1 generation. The effect disappeared in the F2 generation. The prenatal exposure also induced a decrease (26%) in the mean sperm concentration of the F1 that was not detected anymore in the F2 offspring. The CpGs of the H19 CTCF-binding site 2, were analyzed. A correct methylation of H19 CTCF-binding sites is indeed known to be important for the production of mitogen factors controlling fetal growth. The 1st, 2nd and 6th CpGs exhibited significant methylation percentage losses in alcohol-exposed offspring as compared to the same positions in control mice whereas the 3th, 4th and 5th CpG methylation patterns were unaffected. A link can be hypothesized between the hypomethylation of a fraction of the H19 CpGs and the decreased spermatogenesis observed in the alcohol-administered female offspring.

P11.053

Epigenetic signatures of the human extraembryonic tissues reflect the common principles of genome reprogramming during early embryogenesis

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Initiation of tissue-specific differentiation in the early ontogeny is considered as a result of global epigenetic modifications. Studies in model mammals have shown a clear difference in the DNA methylation level between embryonic ectoderm and mesoderm in comparison with primitive endoderm and trophoblast. However, there is sparse information about the mode of epigenetic reprogramming during human development because of tissues unavailability after implantation. The first accessible samples can be obtained from early miscarriages only. We have performed genome-wide DNA methylation analysis of the cytotrophoblast (CT) and extraembryonic mesoderm (EM) from 2 induced abortions, 5 miscarriages with normal karyotype and 6 spontaneous abortions with diploid-aneuploid mosaicism using HumanMethylation27 BeadChip (Illumina, USA) covering 27,578 CpG sites of 14,495 genes. All samples regardless the karyotype and developmental results were clearly separated by two clusters specific to the studied tissues. This finding can be explained by higher methylation of CpG-sites in EM (16,450) vs. CT (10,138). Differentially methylated CpGs were observed in 4,898 genes (FDR<0.01, P<0.01). To determine the biological relevance of these genes a Gene Set Enrichment Analysis using hypergeometric testing for KEGG pathways and Gene Ontology categories was performed. The selected genes were related to cytokine-cytokine receptor interactions (120 genes, P=0.0003), neuroactive ligand-receptor interactions (116 genes, P=0.0005), cell-cell signaling (275 genes, P=0.0002), cellular homeostasis (275 genes, P<0.0001), and others. Our results provide first evidence for the significant impact of epigenetic modifications into tissue specificity and confirm the common principles of genome reprogramming. This study was supported by Federal Program P806 and P1161.

P11.054

Tissue specific epigenetics mechanisms of the I.4 promoter of the CYP19A1 gene in human adipocytes

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Background: Aromatase encoded by the gene CYP19A1, catalyses the synthesis of oestrogens from androgens. In adipose tissue, basal transcription is in part driven by promoter I.4. We sought to determine whether methylation of CpG sites within this region is associated with aromatase RNA expression.

Methods: Omental and subcutaneous adipose tissue samples were taken from 31 obese subjects undergoing bariatric surgery. DNA and RNA were extracted from purified mature adipocytes. Methylation status of CpG sites was determined using quantitative pyrosequencing (QIAGEN Pyromark Q24 at -350 (I.4.1) and -316 (I.4.2) bases upstream of the transcription start site (TSS) of promoter I.4 and +152 (I.4.3) bases downstream of the TSS within an SP1 binding site. Total aromatase expression was determined using qRT-PCR, with primers binding to the coding region.

Results: There were no differences in methylation at I.4.1 and I.4.3 in either omental or subcutaneous tissue with mean values between 75.8% and 79.2% (range 2.0 - 98.0%). I.4.2 methylation was significantly lower with mean values between 21.9% and 36.8% (range 3.0 - 96.0%). In omental tissue percentage methylation at I.4.1 and I.4.2 were positively correlated with each other and negatively correlated with total aromatase RNA expression (R = -0.516, P = 0.017 and R = -0.522, P = 0.015). In subcutaneous tissue methylation of the I.4.3 was positively correlated with aromatase expression (R = 0.549, P = 0.004).

Discussion: Methylation of CPG sites in the promoter region of human adipose tissue aromatase have both positive (I.4.3) and negative (I.4.1and I.4.2) effects on transcription.

P11.055

DNA methylation and gene expression changes in monozygotic twins discordant for psoriasis: identification of functionally important genes involved in immune response

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Psoriasis is a common chronic inflammatory disease affecting the skin, scalp, nails and joints. Psoriasis has a strong genetic component with ~20 susceptibility loci, but epigenetic changes are involved since concordance rates among monozygotic (MZ) twins are 35-70%.

Here we used MZ twins (n=20 pairs) discordant for psoriasis to explore genome-wide differences in DNA methylation and gene expression. The study of discordant MZ twins is an attractive model to investigate epigenetic mechanisms in disease. Discordance in this context can be interpreted as a result of external factors that shape the epigenetic profile and thereby the susceptibility for disease through altered gene expression. We isolated different lymphocyte subpopulations and studied single-cell types (CD4+ and CD8+) to overcome the issue of epigenetic heterogeneity in whole blood. In order to detect gene specific DNA methylation differences, we used the 27K Infinium methylation assay (Illumina). To integrate the analysis of global methylation status and gene expression of approximately all associated genes we used HumanHT gene expression assays (Illumina). Analysis of these data identified genes where differences in DNA methylation between twins were correlated with gene expression in CD4+ cells, thus identification of genes where DNA methylation has a functional role in development of psoriasis. We also present preliminary data on genome-wide DNA methylation (RRBS) and ChIP.

To our knowledge this is the first study using MZ twins discordant for psoriasis in order to reveal epigenetic alterations which potentially contributes to the development of disease.

P11.056

Finding the Genetic Cause of Bladder Epispadias-Exstrophy-Complex (BEEC)

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The Bladder Epispadias-Exstrophy-Complex(BEEC) includes urological anomalies with different degrees of anterior midline defects that include epispadias(E), bladder exstrophy(BE), and cloacal exstrophy(CE). The incidence of these conditions varies from 1:30,000 for BE, 1:100,000 for epispadias, to 1:300,000 for CE. Although rare, treatment for these birth defects requires numerous surgeries over the first years of life that can be traumatic and costly. The majority of cases are sporadic, nonsyndromic, with a normal karyotype, and unknown etiology. Although most genetic studies failed to identify specific genes defects that underlie the disorder, there is evidence of strong genetic component. We used array comparative genomic hybridization (aCGH) to find submicroscopic regions of chromosome gains or losses in BEEC. DNA from 6-epispadias, 14-BE, and 3-CE patients were analyzed using a 3x720K aCGH from NimbleGen. Analysis was performed with Nexus Copy Number and SignalMap software. Each

suspected CNV (copy number variation) was validated by Taqman-QPCR. Sequence analysis of selected genes was then performed. We identified microduplications in chromosome 7 that include genes important in urinary tract development, such as HOXA1-11(7p15.2) and a novel gene PHF14 (7p21.3). Also in other affected patients, we found several microdeletions in novel genes located at chromosome 13(13q14.2 and 13q32). Additionally, we found CNV-losses in the developmental genes DLG2(11q14.1) and NOTCH2(1p12) that display interesting expression profiles in the genitourinary system. Further studies of these genes and their expression profiles will help us to understand the genetic cause of BEEC that may lead to advances in the diagnosis, counseling, and treatment of these patients.

P11.057

The effect of sample mixups in genome-wide studies

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Sample mix-ups can arise during sample collection, handling, genotyping or data management, and might account for part of the missing heritability of complex diseases. However, it is unclear how often sample mix-ups occur in genome-wide studies, as there are no post-hoc methods that can identify these mix-ups in unrelated samples. We have therefore developed an algorithm (MixupMapper) that can both detect and correct sample mix-ups in genome-wide studies by looking at gene expression levels. Our method utilizes the principle that some genetic variants strongly affect expression phenotypes, permitting us to check for every sample whether its phenotypic measurements correspond to the genetic variants that affect these phenotypes.

We show that sample mix-ups are common in genome-wide studies by applying our method to five publicly available human datasets on the genetics of gene expression. On average, 3% of all analyzed samples had been assigned incorrect expression phenotypes; in one of the datasets 23% of the samples had incorrect expression phenotypes. The consequences of these sample mix-ups are substantial: when we corrected these sample mix-ups, we identified on average 15% more significant cis-expression quantitative trait loci (eQTLs). In one dataset, we identified three times as many significant cis-eQTLs after correction.

Through simulations we show that a low percentage of sample mix-ups may lead to an underestimation of the explained heritability of complex traits in genome-wide datasets as well. Sample mix-ups should therefore call for serious attention in past and future genome-wide studies.

P11.058**

Disease gene identification by exome sequencing

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Classical positional cloning techniques for disease gene identification have benefited from the recent advancements of next generation sequencing technology. In this presentation we discuss three whole exome sequencing strategies for disease gene identification in rare and sporadic disorders where classical genetic approaches do not apply.

1) Rare sporadic disorders lack large pedigrees due to reduced fecundity. For this we hypothesize that de novo mutations in a single gene are the underlying genetic cause. We resolved the genetic cause in two of such disorders (Schinzel-Giedion syndrome and Bohring syndrome) by identifying rare coding variants that affect the same gene in multiple patients.

2) In case of more frequent (genetically heterogeneous) sporadic disorders, the underlying cause might be attributed to single de novo mutations in a large mutational target. The amount of patients required to find an overlap however increases exponentially with the amount of disease loci. By sequencing a patient as well as the unaffected parents we identified de novo mutations in 6 out of 10 patients as the potential cause for mental retardation.

3) When dealing with a rare recessive monogenic disorder the identification of the disease gene is intrinsically easier because the search is for two independent events in a single gene. We have shown for patients with Sensenbrenner syndrome that a single patient and a sufficient amount of control data can suffice to determine the cause of the disease.

We conclude that exome sequencing allows robust and unbiased disease gene identification in both rare and common genetic disorders.

P11.059

A common micro-deletion in intron 2 of the human GLA gene identified by a fast PCR - Sanger sequencing workflow

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Aberrations in the X-linked human GLA gene cause Fabry disease and a large number of mutations have been described. Given this multitude of mutations the only comprehensive mode of detection is DNA sequencing of the exons and intron-junctions.

During testing of published primer pairs for amplification and subsequent sequencing of the seven exons in the GLA gene I have found that a micro-deletion of the pentamer CAGCC is frequently found upstream of exon 3 in the bona fide healthy population. Of the 95 specimen of a “normal” human DNA control panel tested, 79 were found to be homozygous intact, 11 were heterozygous for the deletion and 5 were homozygous carriers of the deletion. The significance of this unusually frequent micro-deletion is unknown; does it point to a “mutational faultline” in the GLA gene ?

The poster also describes a novel, fast workflow for re-sequencing exon/intron portions of the human genome: fast PCR (ca. 50 min), followed by amplicon purification using preparative e-gel electrophoresis (ca. 20 min) and subsequent fast Sanger cycle sequencing (ca. 40 min), electrophoresis on an Applied Biosystems capillary Genetic Analyzer (ca. 60 min) finalized by sequence detection using Variant Reporter™ data analysis software.

P11.060

New approaches for Familial Hypercholesterolemia (FH) genetic diagnosis

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Familial Hypercholesterolemia (FH) is an autosomal dominant disorder caused by mutations in the *LDLR*, *APOB* and *PCSK9* genes. Classically, FH mutation detection is carried out by Sanger sequencing and MLPA, since Copy Number Variations (CNVs) account for 5-10% of *LDLR* mutations.

We have developed the LIPOchip® tool, a DNA-array that detects point mutations and CNVs simultaneously and has been used to diagnose more than 5,000 index cases. LIPOchip® current versions detect more than 80% of the Spanish, Dutch, Italian and British mutations but, due to the LDLR heterogeneity and the lack of frequency studies, LIPOchip® is not viable worldwide.

Two technical approaches have been tested to produce a new, rapid, economic and universal FH genetic diagnosis tool: the Affymetrix resequencing chip and the Next Generation Sequencing Genome Sequencer Roche GS Junior. We have developed protocols to allow these tools detecting substitutions, small indels and CNVs in the *LDLR*, *APOB* and *PCSK9* genes.

Our first results revealed the potential of our Affymetrix array design in detecting FH causing variants (even small indels and CNVs). However, there are two main issues to improve: the number of no calls and the robustness of the results.

Regarding the GS Junior, the accuracy of the obtained results was high and we have managed to sequence the whole *LDLR* gene and target exons of the *APOB* and *PCSK9* genes. CNV detection was also analyzed and we expect to reach a routine tool able to analyze 40 patients by run, obtaining their final result in a week.

P11.061
Multiplexed targeted next-generation sequencing as a comprehensive mutation screening approach for Fanconi anemia

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Background: Fanconi anemia (FA) is a recessive chromosomal instability syndrome characterized by congenital malformations, bone failure, and susceptibility to malignancies. Mutations in 15 genes have been shown to cause FA. Since FA patients require special treatment, and syndromes with overlapping clinical symptoms exist, molecular diagnosis is required. The current screening approach is neither time- nor cost-effective and may miss various aberrations, such as large deletions. Here we evaluate the use of a multiplexed targeted next-generation sequencing (NGS) approach in the diagnosis of FA.

Methods: We selected samples from 12 FA patients with various hetero-/homozygous variations including large deletions and small deletions/insertions. For one sample, only one pathogenic mutation had been found by Sanger sequencing. We used a custom Agilent in-solution SureSelect kit to enrich for the known FA genes. The 12 resulting sequence libraries were tagged with a unique barcode and pooled before sequencing 74 cycles paired-end in one lane on the Illumina GAIIx platform. An in-house variation detection pipeline, including a tool for large deletion detection, was used to score for relevant mutations.

Results: An average of 2.8 million unique reads were obtained per sample, resulting in a median coverage of ~100 fold. All disease causing mutations have been detected in our validation panel. In addition, a novel large deletion mutation in an unresolved case has been detected and validated through conventional sequencing.

Conclusion: Multiplexed targeted NGS offers a fast and reliable method for molecular

diagnosis, without the need for preselection by complementation studies.

P11.062
Transcriptional regulation of Fanconi anemia (FA) genes containing NLS-encoding sequences controls nuclear FA pathway activity

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The study of the FA/BRCA pathway in the last decade has added increasing insight in the function of individual FA proteins as well in their role in DNA damage repair. Few studies, however, have focused on the transcriptional regulation of the genes themselves. Since the FA pathway plays a role in the cell cycle, one can expect that (a subset of) the FA genes will be cell-cycle regulated. The aim of this study is to determine the behavior of all FA genes during the cell cycle combined with an *in silico* analysis of their promoter elements.

We could separate the pathway genes into two groups based on their mRNA expression during the cell cycle and/or response to serum in the human glioblastoma cell line T98G. The responsive group consists of four core-complex members (*FANCA, -B, -E*, and *-M*), the ID-complex members (*FANCD2* and, *-I*), and the downstream genes *BRCA2*, and *-J*. The non-responsive group consist of the subcomplex partners of *FANCA*, *-G*, of *FANCB*, *-L*, of *FANCE*, *-C* and *-F*, and of *BRCA2*, *PALB2*. The promoters of each group were analyzed to identify common transcription factor models with Genomatix. For both groups, models containing homeodomain TF families, known to be involved in development were identified. The responsive genes are furthermore characterized by SP1 and E2F sites in the vicinity of their transcription start sites.

The responsive genes contain Nuclear Localization Signals (NLSs)-encoding sequences, suggesting that these constitute the driving forces for nuclear import when necessary during DNA replication.

P11.063
Applying Massive Parallel Sequencing to Molecular Diagnosis of Marfan and Loeys-Dietz Syndrome

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The Marfan (MFS) and Loeys-Dietz (LDS) syndromes are caused by a wide variety of mutations in the Fibrillin 1 (FBN1) and Transforming Growth Factor Receptor 1/2 genes (TGFBR1/2), respectively. With conventional mutation screening technologies, analysis of this set of genes is time-consuming and expensive.

We have optimized a cost-effective and reliable mutation discovery strategy using Next Generation Sequencing technology. In a first phase, five MFS or LDS patient samples with previously identified mutations and/or polymorphisms in FBN1 or TGFBR1/2 were amplified using a multiplex PCR reaction and sequenced on a Genome Sequencer FLX (Roche). All expected mutations could be identified. In a second phase, we validated the technique on 87 samples from MFS patients fulfilling the Ghent criteria using two GS-LR70 runs and one Amplicon Titanium XLR70 run (Roche). To differentiate the samples within a single run, we have used up to 30 different Multiplex Identifiers (MIDs). This resulted in the identification of 66 different FBN1 mutations in a total of 74 patients. Multiplex Ligation-dependent Probe Amplification (MLPA) analysis of the remaining 13 negative samples identified one duplication and two deletions in FBN1. For the remaining 9 negative samples, Sanger sequencing of the coding sequences which were investigated with NGS, did not reveal additional variants.

Our data support that multiplex PCR of all coding exons of FBN1 and TGFBR1/2 followed by Next Generation Sequencing analysis and complemented with MLPA, is a clear-cut strategy for a time- and cost-effective identification of mutations.

P11.064
Evaluation of single-step homogenous screening assays for rapid detection of *FMR1* CGG repeat expansions

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Fragile X syndrome is one of the most common genetic causes of mental retardation, second only to Down syndrome. It is predominantly caused by hyperexpansion (>200 repeats) and hypermethylation of CGG repeats in the *FMR1* promoter region, which results in gene silencing. Smaller unmethylated expansions in the gray zone and premutation range (45 - 200 repeats) have been associated with premature ovarian failure (POF) and fragile X-associated tremor ataxia syndrome (FXTAS). We previously developed several methylation-specific and direct triplet-primed PCR based assays for analysis of the *FMR1* CGG repeat structure and expansion. We have now adapted these assays for single-step homogeneous screening of *FMR1* CGG repeat expansions by melting curve analysis in the presence of SYBR Green I nucleic acid dye. Melting peak profiles from 5'-anchored and 3'-anchored triplet-primed PCRs were evaluated. For 3'-anchored triplet-primed PCRs, the melt peak profiles or normal and expanded alleles were distinctly different. In contrast, 5'-anchored triplet-primed PCRs produced similar melting peak morphologies from normal and expanded alleles, although melting peak temperatures from expanded alleles were slightly increased compared with normal alleles. Therefore, 3'-anchored triplet-primed PCRs are more sensitive compared with 5'-anchored triplet-primed PCRs in distinguishing normal from expanded alleles by melting curve analysis. This difference likely stems from the position(s) of the AGG interruption(s) in most expanded alleles. We conclude that melting curve analysis represents a viable single-step strategy for rapid screening of *FMR1* CGG repeat expansions.

P11.065
Evaluation of MGMT gene promoter methylation by comparing qMS-PCR and MS-HRM analysis in GBM

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Turkey.
Current therapeutic options for patients with glioblastoma multiforme (GBM) consist of surgical resection followed by radiation and chemotherapy. Despite this aggressive multimodality approach, patients continue to have a poor prognosis, indicating the need for an individual molecular drug therapy for each case as a novel therapeutic modality. Expression of the O(6)-methylguanine-DNA methyltransferase (MGMT) gene has shown to correlate with the clinical outcome in GBM patients treated with alkylating agents. HRM is an extended melting curve analysis method, but it requires additional analysis software and special reagents. The aim of the study is to evaluate the effectiveness of qMS-PCR and MS-HRM, two distinctive methods for identifying methylation in GBM patients. We evaluated the status of MGMT gene promoter methylation in 20 primary glioblastoma patients and 2 GBM cell lines (U-87MG, U-118MG) and analyzed the correlation between qMS-PCR and MS-HRM methods by individually designed primers and probes.
In qMS-PCR, 5 cases were found to be unmethylated. According to the analysis of the MS-HRM results; 3 of the 5 cases were correlated with qMS-PCR and 2 cases were heterogenous. Methylation status was correlated in both cell lines, 55-65% methylated and 75-85% methylated groups in these two analysis.
The results indicated the importance of determining individual genetic differences by using both qMS-PCR and MS-HRM analysis for inactivation of MGMT is indispensible.

P11.066
Whole transcriptome expression microarray profiling of cancerous and adjacent normal total RNA

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Whole transcriptome expression profiling by microarray analysis is an important tool for classifying tissue and tumor types and understanding biological mechanisms. This profiling measures the alternative splicing of exons from RNA leading to the translation of different proteins. To address the need for whole transcriptome expression profiling we have developed an exon workflow which includes catalog and custom microarrays, a whole transcript labeling method, and data analysis software. The Low Input Quick Amp Whole Transcriptome Labeling Kit employs a mixture of random nucleotide and oligo dT-based T7 promoter primers resulting in high cRNA yields and Cy-labeled specific activities from nanogram amounts of RNA. The probes on the SurePrint G3 microarrays were designed from the high quality content of public databases including RefSeq and Ensembl. Exon arrays were analyzed for gene level and exon level expression using GeneSpring GX 11.5 software, thus enabling whole transcript profile comparisons within two days. Comparisons of technical replicate samples demonstrate high reproducibility with wide dynamic range across a broad range of RNA input. High correlations were also demonstrated in comparisons to orthogonal expression measurement techniques including RNA-Seq. The whole transcriptome workflow was used to detect alternative splicing of exons between cancerous and normal cells resulting in gene and exon expression profiles consistent with the current literature. SurePrint G3 microarrays were also used to assess differential expression of non-coding RNAs (lincRNAs) in these samples. The combined results demonstrate the value of identifying known exons and showcase the importance of including non-coding RNA profiling in cancer research.

P11.067
Using HGNC to improve and expand Gene Family resources

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The HUGO Gene Nomenclature Committee (HGNC) has assigned unique approved gene symbols and names to over 30000 human loci, over 19000 of which represent protein coding genes. We also name pseudogenes, phenotypic loci, genomic features and non-coding RNAs. Our website: genenames.org is an online repository of HGNC-approved gene nomenclature and associated resources including links to genomic, proteomic and phenotypic information, as well as dedicated Gene Family pages.
Approved gene symbols are based on names describing the structure, function or homology and the HGNC organise genes into gene groups and gene families where possible. Previously the HGNC has created dedicated Gene Family web pages many of which have specialist advisors that both check their accuracy and help to maintain them. However, these pages currently only represent a subset of human gene families. We now intend to expand and generate more Gene Family pages using data curated by the HGNC combined with external resources that specialise in particular gene families. These new Gene Family pages will be sorted into meaningful categories including functions, structure, homology or a mixture. We will provide links from our Symbol Reports to our improved Gene Family resources.
If you have a gene family that you think should be represented, contact us hgnc@genenames.org.
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P11.068
Genomic imprinting of canine *MAS1*, a component of the renin-angiotensin system

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Genomic imprinting occurs in mammals and refers to parent of origin specific epigenetic modifications of genes. The consequence of these modifications is that the two alleles of imprinted genes are differentially expressed. Although few in number, the function of imprinted genes is essential for normal growth and development, and they also regulate aspects of adult behaviour. Aberrant regulation of imprinted genes contributes to a variety of clinical conditions in humans, including somatic undergrowth and overgrowth, behavioural disorders and cancer.
Imprinted genes have mostly been studied in mice and humans. For comparative purposes, and because the dog is now considered an excellent model of human disease, we examined genomic imprinting in the canine. For canine orthologs of three well-characterized imprinted genes (*IGF2*, *H19* and *IGF2R*), we have shown parent of origin specific effects on expression. Imprinted genes usually occur in genomic clusters that are co-regulated by a common imprint. The *MAS1* gene lies upstream of *IGF2R* in mammals and its imprint status in humans is somewhat controversial. Here we report that canine *MAS1* is imprinted, with expression of the maternally-derived allele in several fetal tissues and in neonatal umbilical cord. The protein encoded by *MAS1* is a component of the renin-angiotensin system, a complex regulatory network that is essential for normal human physiology and is altered in several disease states. If *MAS1* (or other RAS components) is imprinted in humans, this could have implications for human health and disease. We suggest that the imprint status of human *MAS1* should be re-evaluated.

P11.069**
Towards a comprehensive overview of genomic variation in patient-parent trios

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We performed exome sequencing on 10 patient-parent trios to obtain a comprehensive overview of genomic variation. These patients were selected based on the presence of unexplained mental retardation in the absence of *de novo* or rare Copy Number Variations (CNVs) and syndromic diagnosis.
Initial array data (250k) provided a low-resolution insight in the patients common CNVs (on average 3, with an average size of 648 kb). Furthermore we detected on average 7,098 common (based on dbSNP130), 100 uniquely inherited, and 1.2 *de novo* Single Nucleotide Variants and 1,953 Insertions/Deletions (InDels) per patient.
Currently, different algorithms are being tested for the detection of Structural Variation (SV), including CNVs and inversions, and their concordance with high-resolution array data (Affymetrix 2.7M arrays) which will provide a detailed overview of the larger SVs. In combination with the extensive analysis on InDels and SNVs, our analysis will provide a comprehensive overview of the genomic variation within an individual, including *de novo* and inherited variants.

P11.070***

G2P Knowledge Centre: An integrated genotype-phenotype data access portal and online collaborative network

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The unprecedented quantity and complexity of genotype-phenotype (G2P) data currently being generated by research and diagnostic labs worldwide, together with a shift toward online data dissemination and collaborative work patterns, has spawned the need for new data handling, integration and online community workspace tools. To tackle data handling issues, the GEN2PHEN Project has spearheaded the creation of a hybrid federated-centralised database network where disparate G2P data (for both Mendelian traits, and data from genome-wide association studies (GWAS)) are held in multiple distributed databases. These independent databases are then interlinked using common exchange formats and data standards.

The 'G2P Knowledge Centre' (http://www.gen2phen.org) leverages this broad yet complex network, and enables valuable distributed G2P data to be quickly located through an intuitive yet powerful holistic search tool. Allied to the data search is the provision of a user annotation system whereby any user may leave comments or feedback on any search result, dataset or resource. These annotations are then made available to the original data provider and the wider G2P community, adding real value to the data and a second-level peer-review system. To further foster an online collaborative G2P community, the 'G2P Knowledge Centre' provides discrete online workspaces (Interest Groups) as well as blogs, news items, events listings, training materials and full access to the many outputs of the GEN2PHEN project.

P11.071

GNAS NESP and XL hypermethylation in pseudopseudohypoparathyroidism patients.

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Endocrinopathies in patients with hypocalcemia and hyperphosphatemia that share resistance to parathyroid hormone (PTH) are named pseudohypoparathyroidism (PHP). Most PHP types are caused by defects in GNAS, an imprinted gene locus consisting of maternal, paternal and biallelic transcripts. PHPIb patients have isolated PTH resistance and GNAS epidefects while PHPIa presents with hormonal resistance and characteristic physical features such as mental problems, brachydactyly, shortness, round face and subcutaneous ossifications, jointly termed as having Albright's Hereditary Osteodystrophy (AHO) due to maternally inherited GNAS mutations. PPHP patients have AHO without hormone resistance and paternally inherited GNAS mutations. This classification of PHP was made years ago but was recently questioned since GNAS epidefects were also identified in PHPIa. Our present study further complicates this (epi)genotype/phenotype relation as we found for the first time GNAS epidefects in PPHP. We quantified 3 differentially methylated regions in GNAS: NESP (2 regions of 13 and 20 CpGs), XL (24 CpGs) and exon A/B (20 CpGs) methylation via the Sequenom EpiTYPER in 3 PPHP patients with variable degree of AHO (shortness, brachydactyly, behavior problems, round face) but no GNAS coding mutation. These patients showed significant hypermethylation of preferential CpGs in NESP and XL (Z-test, p<0,05) and one patient also had significant A/B hypermethylation. This is a novel imprint pattern that has not been described since PHPIb/PHPIa epidefects are characterized by A/B and XL hypomethylation and NESP hypermethylation. Further studies are needed to unravel this novel methylation pattern in relation to an AHO phenotype by including more patients.

P11.072

Noninvasive detection of GSTP1 gene in urine samples from prostate cancer patients

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Purpose: Methylation of the GSTP1 gene promoter region is the most common epigenetic change in prostate carcinogenesis. In our study, we want to analyse the methylation status of gene GSTP1 in urine samples from prostate cancer (PCa) patients, as a noninvasive method to detect PCa from urine.

Material and method: We collected voided urine specimens from 75 men who underwent transrectal ultrasound-guided biopsy of the prostate for suspected malignancy, and from 58 men with benign prostatic hyperplasia (BPH), representing the control subjects. We isolated genomic DNA from urine specimens and after, subjected them to sodium bisulfite modification.

Analysis of the methylation status of GSTP1 gene was done by methylation-specific PCR (MSP) method, and we correlated them with Gleason score and PSA levels.

Results: Using methylation-specific PCR (MSP) method, we found GSTP1 gene hypermethylated in 69 of 75 (92 %) PCa patients , and in 5 of 58 (8.62%) patients with BPH.

Conclusion: From the obtained results we can conclude that, the detection of aberrant methylation of gene GSTP1 in urine may offer a promising approach for the noninvasive diagnosis of prostate cancer.

P11.073

Molecular Diagnosis of the BRCA genes in Hereditary Breast and Ovarian Cancer families, using Roche/454 Pyrosequencing technology.

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Screening for deleterious mutations in the *BRCA* genes is an essential service to women with familial risk of breast or ovarian cancer. To offer this service as widely as possible, it is necessary to develop faster and less expensive methods. New high-throughput genotyping technologies give high-quality sequence data with quick turnaround and low cost. We evaluated Roche-454 technology for genetic diagnosis.

Sixty-four familial breast cancer cases were analyzed by amplicon pyrosequencing and Sanger dideoxy sequencing, plus 14 by pryosequencing only.

Pyrosequencing was validated for up to 97 % of amplicons in each of four standard runs of 16 cases, and up to 98 % using Titanium chemistry. A minimum coverage depth of 40 reads was required to validate an amplicon as 'analysed'. All variants detected by dideoxy sequencing were also detected by pyrosequencing, including eleven deleterious mutations.

Six of the 84 amplicons contained homopolymer tracts in the coding sequence systematically misread by the Amplicon Variant Analysis software. Development of in-house software for the reanalysis of homopolymer data showed that most misread sequences were the result of intermediate pyrosequencing signals: histograms of read data aided the analysis considerably and allowed validation of the majority of homopolymers.

This pilot study on the *BRCA* genes indicated that pyrosequencing was as sensitive as dideoxy sequencing, and the effort and cost involved were comparable. Moving to Titanium chemistry, with more and longer reads and the multiplexing of samples, permits analysis of 64 or more samples simultaneously, giving pyrosequencing an advantage over classic techniques.

P11.074***

An inflammasome genome screen to detect novel disease genes in hereditary autoinflammatory disorders.

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Hereditary autoinflammatory disorders, for example Familial Mediterranean Fever (FMF), and the cryopyrinopathies (CAPS) are caused by mutations in 'inflammation-related genes', which include genes in which a mutation can trigger an uncontrolled, aimless activation of the inflammatory process in response to innocuous stimuli.

A large number of patients with autoinflammatory diseases, remain

genetically unexplained. Several studies have shown that aberrant activation of the IL-1β pathway via the inflammasome is a common mechanism in the pathogenesis of autoinflammatory diseases. We hypothesize that mutations in a variety of genes involved in the control of inflammasome function can result in autoinflammatory disease in a specific patient.
To test this hypothesis an 'inflammasome genome' was designed. This 'inflammasome genome' consists of 120 genes known to be involved in, or associated with proteins functioning in the inflammasome. Next Generation Sequencing was used to efficiently screen the 'inflammasome genome' in 60 selected patients, for novel disease associated variations. A combination of 60 barcoded-patient-DNA-libraries was pooled and enriched on a single custom Agilent CGH 1M array followed by SOLID sequencing. Sanger Sequencing confirmed 50 novel candidate disease associated variations found in 34 different patients.
We are currently in the process of functionally testing the identified candidates for their effect on inflammasome function in human cells to determine their implication in the disease. The identification of new disease-causing genes are expected to not only help diagnostics but also future therapies. Moreover, this study will shed more light on the function of various inflammasomes, which are still poorly understood.

P11.075
Identification of the causative gene for SPG27 by Exome capture

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Hereditary spastic paraplegia (HSP) is a motor neuron disease for which the key symptoms are lower limb spasticity and weakness because of progressive neurodegeneration events. The group of HSP is particularly heterogeneous because different modes of inheritance were observed (dominant, recessive and X-linked) as well as various levels of symptoms complexity; pure and complicated forms were described. To date, over 45 spastic paraplegia locus were identified and 20 genes identified. A previous linkage analysis on an autosomal recessive pure HSP family identified a new locus (*SPG27*) on the 10q22.1-10q24.1 region; a locus partially overlapping the *SPG9* locus. We proposed to perform use an exome capture approach with the Agilent Technology SureSelect Human All Exon Kit (50MB) and proceed to resequencing with ABI SOLID4 DNA sequencer. Four affected individuals, the unaffected-carrier father and another unaffected sibling carrying the same disease-allele as the father will be used. As we may be looking for compound-heterozygote mutations, we will develop an algorithm to determine the parental origin of variants and the more promising mutations will be validated through Sanger sequencing. By this method, it will be possible to cover the 40 genes, find the causative mutation and maybe solve the *SPG9* clue.

P11.076
Integration of GWAS data using Gene Ontologies and "2-step analysis" to select disease genes candidates for HSCR.

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Hirschsprung's disease (HSCR) is a congenital malformation of the hindgut with low sex-dependent penetrance, variable expression and involvement of one or more genes.
One of the most innovative tools for the identification of additional genomic regions associated with HSCR is a Genome Wide Association study (GWAS). An International Consortium (ICHSCR) was settled in 2004 to achieve this goal. Using this strategy, several loci have been described as "HSCR-associated regions" and their "HSCR gene". We propose a new approach based in an innovative data processing of the results obtained from a GWAS performed on 213 S-HSCR trios worldwide recruited by ICHSCR. Such approach leads the identification of specific terms from the Gene Ontology database (GOs) associated with the pathogenesis of HSCR. We have used a new bioinformatic tool: http://gesbap.bioinfo.cipf.es, to compare Spanish patients (reference group) with the rest of populations (Consortium) from ICHSCR through 2 consecutive methods:
1) Using the p value (<0.05) of the GOs obtained from gesbap from both populations, we got 17 significant GOs in Spanish population of which just 11 were significant in all the populations.
2) "2-step functional analysis": we calculate the adjusted p value from the nominal one applied to Consortium populations. Results: 27 significant GOs in all the populations (Spanish and Consortium).
We have established a new tool to select a wide spectrum of genes as candidates in the context of HSCR. Further analyses are needed to elucidate the complex nature of HSCR.

P11.077
Immunostaining of acetylated histone H3 on metaphase chromosomes reveals band-specific patterns differing in pre- and postnatal human development

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Acetylation of 9th lysine residue in histone H3 (AcH3K9) is an epigenetic modification of human genome involved in gene regulation and generally associated with transcriptionally active, or potentially active, chromatin.
We aimed at analysis of acetylated histone H3 distribution in metaphase chromosomes from PHA-stimulated fetal cord blood lymphocytes compared to that from adult lymphocytes. Study was performed on 79 metaphases from 3 embryos (20-24-weeks gestation) and 60 metaphases from 5 adults fixed with 2% acetic acid in ethanol.
Distribution of AcH3K9 on metaphase chromosomes was studied by indirect immunofluorescence with specific monoclonal antibodies against AcH3K9 (Abcam, USA). Chromosomes were identified by centromeric index and DAPI banding. Immunostaining of metaphase chromosomes revealed regions of different fluorescence intensity, with most bright fluorescence in R-bands and absence of signal in pericentromeric heterochromatin.
Signal distribution in chromosomes from fetal lymphocytes demonstrated difference in fluorescence intensity between homologous chromosomes in bands 1p36.3, 2p23, 2q33, 10p11.2, 11p15. These differences may be determined by genomic imprinting.
Five bands - 5p13, 5p15, 9q13,16p13,18p11.32 - showed differences in AcH3K9 level between fetal and adult chromosomes. Bands 5p13, 5p15and16p13 were hypoacetylated; bands 9q13and18p11.32 demonstrated higher acetylation level in fetuses compared to adults.
Thus, pattern of AcH3K9 distribution along metaphase chromosomes of both fetal and adult lymphocytes corresponds to a band-specific manner, resembling R-banding. Differences in AcH3K9 of some bands between fetal homologous chromosomes as well as between fetal and adult chromosomes might be attributed to stage-specific epigenetic patterns in the same cell types of humans.
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P11.078
Holoprosencephaly and Notch signalling pathway: transcriptomic approach using chick model

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Holoprosencephaly (HPE) is a congenital malformation of the human brain due to an imperfect division of forebrain during early development. It is now currently admitted that HPE is a multihit pathology caused by at least two or more dysfunctional events involving genes from more than one signalling pathway.
The Shh and Nodal pathways have been presented to be the main signalling pathways involved in human HPE. To a lesser extend, FGF

has also been identified to be implicated. Importantly, by CGH array we have recently identified a component of the Notch pathway, *Dll1*, as a new HPE candidate gene. This result suggests that a dysregulation of Notch pathway may well confer a susceptibility to HPE.
However, if previous studies have showed the importance of Notch throughout the neurogenic phase of forebrain development, a link with HPE has never been described. To assess this question, we have implemented an *ex ovo* chick embryos culture system with a pharmacological inhibitor known to block specifically Notch pathway. Subsequently, the molecular reprogramming of the treated brain has been tested using expression microarray and *in situ* hybridization approaches. These analyses provide functional data about the Notch pathway during HPE appearance as well as throughout normal forebrain development.

P11.079

Mutations in KLF1 as a cause of Hereditary Persistence of Foetal Haemoglobin

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Reactivating the foetal haemoglobin (HbF) program of development to raise the level of HbF in sickle cell disease or β-thalassemia patients would greatly ameliorate the symptoms of these diseases. This can only be achieved by first understanding fully the genetic switch from foetal to adult haemoglobin. Substantial progress has recently been made in this field, however the exact mechanism remains elusive. Using a combination of clinical research coupled with basic research using state-of-the-art technologies generated data and knowledge that addresses parts of this intricate mechanism. A unique and large Maltese family with elevated levels of HbF was identified through the National Screening Program conducted in Malta. An extensive molecular study revealed a novel mutation in the human EKLF/KLF1 transcription factor that was responsible for an overall dominant HbF increase in this family from Malta. Gene expression array analysis delivered a list of KLF1-target genes that might also be implicated in the globin biosynthesis pathway. The same study showed how BCL11A is developmentally regulated by KLF1 and that diminished levels of BCL11A as a result of KLF1 haploinsufficiency resulted in higher HbF levels. A different Maltese family carrying the same mutations has recently been identified, but these individuals displayed much lower foetal haemoglobin levels. This suggested the presence of modifier genes strongly affecting the Hereditary Persistence of Foetal Haemoglobin (HPFH) phenotype in conjunction with the KLF1 mutations and therefore we device methods at the genomic, transcriptomic and proteomic levels to identify these modifier genes acting in conjuction with KLF1.

P11.080

Re-Sequencing of human samples: comparing and combining different libraries, sequencing technologies and software tools

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Strategies for sequencing human genomes and transcriptomes need to be designed based on the goals of the project.
Genomic libraries with small (300 bp) and large(up to 8 kb) inserts to identify SNPs and rearrangements can be prepared. Different enrichment technologies are applied to sequence specific subsets of genes or the whole exon.
Transcribing RNA into cDNA is another procedure for transcriptome analysis.For a quantitative analysis, UTR libraries are used to generate expression profiles. Regulatory studies are performed withChIP-, and small RNA libraries.
The Illumina HiSeq 2000 provides the industry's highest **sequencing** output, quality and fastest data generation rate for resequencing and quantitative analysis.
Amplicons can be sequenced on the Roche GS FLX or with the classical Sanger technology to obtain longer reads.
With the Pacific Biosciences PacBio RS real time single molecule sequencer, long reads in the range of Sanger sequencing and so-called strobe readscan reveal multiple types of genetic variation, such as large-scale rearrangements, as observed in cancer.
In addition, direct sequencing of methylated and other modified bases will be possible.
Proprietary tools are used for the analysis of Next Gen sequencing data, while third party tools are available for optimised *de novo* assembly or improved hybrid assemblies..
Conclusion
High quality results will be obtained by optimally combining several library preparation methods and the newest Next Gen technologies with state-of-the-art bioinformatics.Thus, the deepest analysis of data and as a consequence, the most robust analysis of the sequence you are working on, will be achieved.

P11.081

A novel mutation in alpha-Actinin-2 responsible for Hypertrophic Cardiomyopathy identified by massively parallel next generation sequencing

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Aim of the present study was to evaluate the capacity of sequence enrichment and next generation sequencing (NGS) to successfully identify mutations in a large family with an atypical variant of hypertrophic cardiomyopathy (HCM). Sequence capture target enrichment and massively parallel NGS might be particularly advantageous in testing for genetically heterogeneous hereditary conditions, such as HCM. Current diagnostic evaluation proceeds by sequencing a large number of genes, based mainly on the relative frequency of the mutations. In more than 35% of patients, the pathogenic mutation is unknown even after very extensive and expensive molecular testing.
We designed a custom in-solution SureSelect enrichment system covering 36 genes. The genes were chosen based on reports of identified mutations in at least one patient. We tested the proband, an 82 year-old man with a mild, asymmetric LV hypertrophy localized to the apex, marked bi-atrial dilatation, restrictive LV filling patter and juvenile onset of atrial fibrillation. After the run on GAIIx and bioinformatics analysis, three variants were identified: TTNchr2:17918115C>T; p.Arg13823Gln ACTN2 chr1:234961223T>C; p.Met228Thr and MYH3 chr11:47313204C>G; p.Ala1198Thr. Of these, only ACTN2 chr1:234961223T>C; p.Met228Thr co-segregated with the disease manifestations in the proband's pedigree .
This is the first study that showed the feasibility of using genomic enrichment by sequence capture followed by NGS to investigate genetic causes of HCM. Such strategy allows simultaneous, efficient and low-cost sequencing of all genes implicated in a particular genetic disorder, with potential implications for other monogenic cardiovascular disorders.

P11.082

Loss of function mutations in *ZBTB24* cause immunodeficiency, centromeric instability and facial anomalies (ICF) syndrome type 2

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Immunodeficiency, centromeric instability and facial anomalies syndrome (ICF; MIM 242860) is an autosomal recessive immune disorder that is characterized by recurrent, often fatal, respiratory and gastrointestinal infections due to greatly reduced serum immunoglobulin levels. About 50% of ICF patients (ICF1) carry mutations in the DNA methyltransferase 3B (*DNMT3B*) gene rendering specific repetitive DNA sequences in their genome hypomethylated. The remaining patients carry unknown genetic defects (ICF2), but share with ICF1 patients similar immunological and epigenetic features including hypomethylation of juxtacentromeric heterochromatin regions. By homozygosity mapping and whole exome sequencing we identified nonsense mutations in the zinc finger and BTB domain containing 24 (*ZBTB24*) gene in four consanguineous ICF2 patients, in an affected sibling pair and in one patient of whom consanguineous descent was unknown.

ZBTB24 belongs to a large family of transcriptional repressors including members with regulatory roles in hematopoietic development and malignancy. By the combinatorial use of FACS analysis and quantitative RT-PCR we demonstrated that *ZBTB24* is ubiquitously expressed in human tissues and cell lines and that the highest *ZBTB24* mRNA levels are observed in human peripheral blood B cell subpopulations, especially in naive B cells. Altogether this argues that ZBTB24 is not only involved in the methylation of juxtacentromeric heterochromatic regions, but that it also regulates the development and/or differentiation of B cells.

P11.083

Implicit conceptual links in the literature: a large untapped reservoir of gene discovery

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The published literature and bioinformatic data are growing faster than individual researchers can track. Text- and data-mining tools have been developed to retrieve explicit links between pairs of concepts (e.g. genes and disease). However, in addition to explicit links, text also contains a complex network of indirect (implied) links containing useful information. We applied concept profiling to probe this vast network of implied information. In a retrospective analysis of 18 gene-disease relationships, implicit information alone could prioritize the causative gene on average within the top 13 out of 200 genes located in a specified linkage interval, at least one year before publication of the landmark paper. The concepts shared between gene and disease enabled the evaluation of the plausibility of the inferred relationship. Of the 40,404,412 possible gene disease pairs, 120,246 (47%, $p < 0.003$) arose exclusively from implicit relations. These results reveal an enormous untapped discovery potential in the implied information of biomedical literature .

P11.084

Next generation sequencing in a family with infantile onset spinocerebellar ataxia identified a novel missense mutation in *C10orf2* gene

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Infantile Onset Spino Cerebellar Ataxia (IOSCA) is a quietly rare disorders characterized ataxia associated with peripheral sensory neuropathy, athetosis, epilepsy, deafness, opthalmoplegia and optic atrophy, and primary hypogonadism in female. Typically, the progressive symptoms developed after upper tract infection in a child with normal developmental milestones until 9-18 months.

In this study, next generation sequencing together with homozygosity mapping identified the disease causing mutation in effected individuals in a Turkish family with spinocerebellar ataxia. Homozygous missense mutation c.1366C>G (L456V) in *C10orf2* gene encoding the Twinkle protein was identified as the disease causing mutation. The open reading frame of *C10orf2* spans five exons and encodes 684 amino acids Twinkle protein, which is a helicase responsible for the replication and maintenance of mitochondrial DNA in mammalian cells. While recessive mutations in the *C10orf2* gene result in IOSCA and hepatocellular encephalopathy syndrome accompanied by mitochondrial depletion, autosomal dominant mutations in the gene cause autosomal dominant progressive external ophthalmoplegia (adPEO) mainly manifesting itself in adulthood.

Pathogenic effect of this amino acid change (L456V) was supported by several lines of evidence. Firstly, c.1366C>G nucleotide change was not detected in 150 Turkish individuals and has not been reported in dbSNP. Secondly, the ClustalW alignment of the amino acid sequence indicated that L456V homozygous missense mutation was at highly conserved position in *C10orf2* shown by comparison to the corresponding sequence of six vertabrates. Lastly, PolyPhen analysis showed that this amino acid change is possibly damaging on the function of the protein.

P11.085

Identification of the *IRXB* gene cluster as candidate genes in severe dysgenesis of the ocular anterior segment.

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PURPOSE: Anterior segment ocular dysgenesis (ASOD) is a broad heterogeneous group of diseases detectable at the clinical and molecular level. In a patient with bilateral congenital ASOD including aniridia and aphakia, a complex chromosomal rearrangement, inv(2)(p22.3q12.1)t(2;16)(q12.1;q12.2), was characterized at the molecular level, to identify candidate genes implicated in ASOD.

METHODS: After negative sequencing of the PAX6, FOXC1, and PITX2 genes, we used fluorescence in situ hybridization (FISH) and Southern blot analysis to characterize the chromosomal breakpoints. Candidate genes were selected, and in situ tissue expression analysis was performed on human fetuses and embryos.

RESULTS: Molecular analyses showed that the 16q12.2 breakpoint in this rearrangement occurs in a 625-bp region centromeric to the IRX3 gene, which belongs to the IRXB cluster. In situ hybridization expression studies showed that during early human embryonic development, the IRX3 gene is expressed in the anterior segment of the eye. Of interest, it has been shown previously that a highly conserved noncoding region (HCNCR) is located 300 kb centromeric to the IRX3 gene and induces, in a murine transgenic assay, an expression pattern fitting that of the IRX3 gene.

CONCLUSIONS: The authors propose that the 16q12.2 breakpoint of this complex translocation is causally related to the ocular anterior segment dysgenesis observed in this patient. This translocation is assumed to separate the HCNCR from the IRXB cluster genes, thus

deregulating the IRXB cluster and leading to the ASOD observed by a positional effect.

P11.086
Genetic analysis of a glomerulonephropathy segregating in a pedigree of French Mastiff

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Human primary glomerulopathies have a high incidence (~6/1000). Several have suspected or confirmed genetic basis. Implicated genes encode structural proteins such as collagen type IV (Alport syndrome) or the laminin β2. A dozen genes are responsible for alterations of podocyte function, of which three involved in juvenile disorders (*NPHS1, NPHS2 and WT1*). Other glomerulopathies have a genetic component but the causative genes are not yet discovered.
Juvenile glomerulonephropathy has been reported in a pedigree of French Mastiffs. Affected dogs develop symptoms during the first year of life with polyuria, polydipsia and signs of azotemia. Diseased dogs are born from clinically healthy parents, without gender predisposition. The proportion of affected dogs within a litter suggests an autosomal recessive mode of transmission. A genome wide association (GWA) study using the 50K canine SNPs Affymetrix array on 30 healthy and 10 affected dogs did not reveal any region significantly associated with the disease. In one chromosome region, however, affected dogs carried one of only two haplotypes over a 4Mb segment. Candidate genes lying within the interval are currently under investigation in conjunction with expression studies in kidney samples from affected dogs versus healthy ones. A new genome wide scan with data coming from the 170K Illumina array is statistically studied at the moment. The genome of two affected dogs (representing the two haplotypes) has been completely sequenced and the resulting traces are being analyzed. RNA-seq experiments of affected and healthy kidney samples are also in progress. Latest results will be presented.

P11.087
Characterization of a novel transcript of the *EHMT1* gene reveals important diagnostic implications for Kleefstra Syndrome

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Kleefstra syndrome is characterized by intellectual disability, childhood hypotonia and a characteristic facial appearance. It can be caused by either submicroscopic deletions of 9qter or intragenic loss of function mutations in the *EHMT1* gene. Until now, routine DNA diagnostic testing for Kleefstra syndrome comprised direct sequencing and MLPA (Multiplex Ligation-dependent Probe Amplification) analysis of the coding region of transcript NM_024757.3. Remarkably, in three patients with a clinical suspicion of Kleefstra syndrome, molecular cytogenetic analysis revealed a submicroscopic interstitial 9q deletion proximal to this transcript. Furthermore, by RT-PCR of the transcript in two of these patients, we found mono-allelic expression of *EHMT1*. in agreement with haploinsuffciency of the gene. We therefore hypothesized that a deletion of regulatory elements or so far unknown coding sequences in the 5' region of the *EHMT1* gene might result in a phenotype compatible with Kleefstra syndrome. Based on *in silico* data, it appeared that the N-terminus of the *EHMT1* gene was recently updated with an extra exon, and that the first untranslated exon of the old transcript is now coding exon 2 (NM_024757.4). MLPA analysis confirmed that in all 3 cases, this novel 5' part of *EHMT1* was deleted. Subsequently, in a panel of 75 individuals without previously identified *EHMT1* mutations, we detected one additional case with a deletion comprising only this 5' part of the *EHMT1* gene and the proximal flanking *C9orf37*.
These results have important implications for the genetic screening of patients with Kleefstra syndrome and further studies of the functional significance of *EHMT1*.

P11.088
Potential use of quantitative expression profiles of mutated Lamin A/C gene as part of the diagnostic work-up of patients with dilated cardiolaminopathies

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Introduction: Diagnosis of dilated cardiolaminopathy, characterized by dilated cardiomyopathy (DCM) and increased risk of arrhythmias, is made after sequencing the *Lamin A/C* (*LMNA*) gene. Current knowledge regarding myocardial expression of Lamin A/C protein and quantitative expression of mutated *LMNA* in myocardium and peripheral blood is very limited. If significant differences in quantitative expression profile exist amongst DCM patients with mutated *LMNA* (DCM-*LMNA*-Mut), DCM patients with wild type *LMNA* (DCM-*LMNA*-WT), and normal control individuals (CTRL), this could be important in diagnostic work-up of cardiolaminopathies.
Methods: We performed immunohistochemistry on myocardial samples from 25 DCM-*LMNA*-Mut patients and on endomyocardial biopsies from 20 normal donor hearts, with anti-Lamin A/C antibodies. Mutated *LMNA* mRNA levels in 20 myocardial and 67 blood samples from DCM-*LMNA*-Mut patients were determined. We examined wild-type *LMNA* mRNA levels in 20 myocardial and 96 blood samples from DCM-*LMNA*-WT patients, and 115 blood samples from CTRL individuals.
Results: Myocardial samples from DCM-*LMNA*-Mut patients have variable loss of protein expression at the nuclear membrane of cardiac myocytes versus DCM-*LMNA*-WT and CTRL samples. *LMNA* was significantly under-expressed in myocardial samples from DCM-*LMNA*-Mut versus DCM-*LMNA*-WT patients. In mRNA from peripheral blood, *LMNA* was significantly under-expressed in DCM-*LMNA*-Mut versus DCM-*LMNA*-WT and CTRL individuals, with most under-expression in splice site, out-of-frame and premature termination codon mutations, followed by missense and in-frame insertion/deletion mutations.
Conclusions: Loss of protein expression of myocyte nuclei, and decreased gene expression in myocardial and peripheral blood mRNA of DCM-*LMNA*-Mut patients, reveal that immunohistochemistry and gene expression could supplement diagnostic work-up for cardiolaminopathies.

P11.089
Genotype instability observed in the long term subculture of LCL samples

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The high-throughput microarray based method for single nucleotide polymorphism (SNP) genotyping has a great advantage in genome wide association studies (GWAS) for identifying human disease susceptibility loci. Although peripheral blood mononuclear (PBMC) is a major resource to collect genomic DNA for genotyping, the Epstein-Barr Virus (EBV)-transformed lymphoblastoid cell line (LCL) is a promising alternative choice to overcome the insufficiency of PBMC. To gain insight into the fidelity of SNP array genotyping in using DNA extracted from LCL, we tested genotype stability of LCL at different propagation stages in terms of cell passages. We genotyped PBMC and LCLs of 20 different individuals using Affymetrix SNP 500k genotyping chips. Six LCL samples for each individual were generated from long-term subcultures up to a passage number of 160. We extensively monitored the genotype concordance for overall SNPs by comparing identity-by-state (IBS) between PMBC and LCL at each propagation stage, by which we were able to profile mismatch rate by chromosome at different propagation stages. Although little difference in genotypes

between PBMC and LCL samples has been reported previously, this study detects slightly decreased concordance between PBMC and LCL samples in old passages. These results suggest that DNA isolated from old passage LCL samples (older than passage number 41) should be avoided for microarray based genotyping.

P11.090
Evaluation of multiple High Resolution Melting (HRM) techniques conducted on three commercially available HRM platforms.

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Introduction: HRM is a genetic screening tool that allows investigators to rapidly detect, identify and focus on samples with relevant mutations before they proceed to expensive and time consuming traditional or next generation sequencing. This study used diverse HRM methods to compare three HRM capable platforms.

Methods: A suite of assays highlighting different HRM techniques, mutation scanning, small amplicon genotyping (SAG) and unlabeled probe genotyping, were optimized on three HRM platforms: the LightScanner® (Idaho Technology, Inc.) / Eppendorf MasterCycler Realplex^2 (Eppendorf), the LightCycler® 480 (Roche), and the 7500 Fast DX (ABI). The assays used LightScanner mastermix and High Sensitivity mastermix that include the double stranded DNA dye LCGreen®Plus (Idaho Technology, Inc.).

Results: All platforms were able to differentiate the mutations in the LIPC (Scanning) and Human THO1 (SAG) assays and detected down to 10% allele fractions in the ADH4 (LunaProbe) assay. All three platforms were able to distinguish between heterozygous and homozygous samples in the CPS1 (SAG) class 4 SNP assay. The LightScanner was also able to differentiate the two homozygous samples. The LightScanner correctly grouped all three genotypes of the OTC class 4 SNP (LunaProbe) assay while the other instruments could not group the heterozygous samples. With the HFE (Multiplex LunaProbe) assay, the LightScanner correctly separated each group of samples while the other platforms could not.

Conclusions: All three systems performed HRM well. The Lightscanner/Realplex platform provided more precise mutation discovery, differentiation and genotyping of difficult target regions including class 4 SNPs.

P11.091
LMX1B expression in tissues and splicing isoform detection

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LMX1B protein is a member of the LIM-homeodomain family of transcription factors, containing two zinc-finger LIM domains and a homeodomain. It is encoded by LMX1B gene (MIM 602575), responsible for Nail-patella syndrome (NPS, MIM#161200), a rare autosomal dominant disorder characterized by a classic clinical tetrad of changes in the nails, knees, elbows, iliac horns and in 40% of cases renal involvement.

A major problem in studying the human LMX1B gene is its scarce or null expression in adult tissues. We were able to detect expression of LMX1B in the cells of hair follicle and urine cells pelleted.

Our previous work demonstrated the presence of two isoforms of LMX1B, differing for seven aminoacids, due to alternative splicing in exon 7. Here we present analysis of different cell types in human and mouse, showing difference of expression of the two isoforms, that could be due to cell specific difference in the mecchanism of splicing and could reflect their differential functional roles.

In order to investigate on the biological role of isoforms, we have investigated difference in cellular localization and evaluated their effect on regulation of trasciptional activity by measure of the luciferase reporter gene, controlled by regulatory sequences of different LMX1B putative target genes, like Podocin, WNT4, PXO2A, PHOX2B, and ACVR1 that we have chosen considering the role of LMX1B in development of kidney, some districts of the central nervous system, and limb.

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P11.092
Analysis of worldwide tandem repeat copy number variation of eight macrosatellite repeats

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Macrosatellite repeats (MSR) comprise a significant proportion of the human genome, usually spanning hundreds of kilobases of genomic DNA. Because of their highly polymorphic nature, MSR represent an extreme example of copy number variation, but their structure and function is largely understudied. This study comprises the genetic analysis of six autosomal and two X chromosomal MSR of HapMap individuals representing Caucasian, Asian and African populations. Tandem repeat copy number variation and stability of the autosomal macrosatellite repeats RS447 (chromosome 4p), MSR5p (5p), FLJ40296 (13q), RNU2 (17q) and D4Z4 (4q and 10q) and X chromosomal CT47 and DXZ4 were investigated. Repeat array size distributions show that these MSR are highly polymorphic with the highest genetic variation among Africans and the least genetic variation among Asians. A mitotic mutation rate of 0.4-1.5% was observed, explaining the high size variability found for MSR. Multimodal distribution patterns show that MSR are organized in a higher order structure. This study extends our knowledge about MSR regarding array size variation and behavior at the population level. Considering that MSR are large repeat arrays, highly polymorphic and can cover large regions of the genome, it is imperative to get a better understanding of their structure and function. Indeed, this extreme form of copy number variation may well explain some of the missing heritability observed in genome-wide association studies.

P11.093
FBN1 gene expression in Marfan Syndrome: a RT-qPCR study.

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Background

Marfan Syndrome (MFS) is caused by defects of the *Fibrillin 1* gene (*FBN1*). We investigated the quantitative expression of *FBN1* mRNA in total RNA isolated from blood samples of MFS patients with *FBN1* mutations.

Methods

Quantitative real-time reverse transcription-PCR was performed in 203 mutated vs. 80 wild type subjects. First-strand cDNA was synthesized from RNA according to the cDNA Reverse Transcription Kit protocol (Applied Biosystems, FosterCity, CA, USA) with three replicas for each sample. Analysis was performed using Applied Biosystems 7900HT Real-Time PCR on *FBN1* cDNA using Hs00153462_m1 probe. Beta actin (*ACTb*, Hs99999903_m1) was selected as the housekeeping gene (HGK). Difference in gene expression was calculated by fold change ratio dividing mean 2^-DeltaCt value in mutated/control individuals. Subgroups were generated based on age and mutation type. Statistical significance was assessed by the Mann Whitney test for unpaired data (alpha=0.05, STATA 10).

Results

FBN1 gene expression declined with age in both cases (31% less in MFS adults than children, p=0.03) and controls (65% less in adults than children, p<0.01). *FBN1* gene showed 53% (p<0.01) and 76% (p<0.01) underexpression in MFS adults and children, respectively vs. age-matched controls, irrespective by mutations type.

	Missense	in-frame Insertion/Deletion	premature termination codon	Splice site
Children (N=95)	55% (p<0.01)	56% (p=0.05)	57% (p<0.01)	61% (p=0.02)
Adults (N=108)	75% (p<0.01)	74% (p=0.03)	77% (p<0.01)	76% p<0.01)

Table 1: *FBN1* underexpression group by mutaton type

Conclusions
Defects of *FBN1* are associated with significantly lower *FBN1* mRNA levels in peripheral blood, irrespective of the type of the mutation in both children and adults.

P11.094
Practical tools to implement massively parallel pyrosequencing of PCR products in next generation molecular diagnostics

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Despite improvements in terms of sequence quality and price per basepair, Sanger sequencing remains restricted to screening of individual disease genes. The development of massively parallel sequencing (MPS) technologies heralded an era in which molecular diagnostics for multigenic disorders becomes reality. Here, we outline different PCR amplification based strategies for the screening of a multitude of genes in a patient cohort. We performed a thorough evaluation in terms of set-up, coverage and sequencing variants on the data of 10 GS-FLX experiments (over 200 patients). Crucially, we determined the actual coverage that is required for reliable diagnostic results using MPS, and provide a tool to calculate the number of patients that can be screened in a single run. Finally, we provide an overview of factors contributing to false negative or false positive mutation calls and suggest ways to maximize sensitivity and specificity, both important in a routine setting.
By describing practical strategies for screening of multigenic disorders in a multitude of samples and providing answers to questions about minimum required coverage, the number of patients that can be screened in a single run and the factors that may affect sensitivity and specificity we hope to facilitate the implementation of MPS technology in molecular diagnostics.

P11.095
Chromothripsis as a mechanism driving complex de novo structural rearrangements in the germline

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A variety of mutational mechanisms shape the dynamic architecture of human genomes and occasionally result in congenital defects and disease. We have developed an efficient experimental and bioinformatics platform to systematically analyze de novo and inherited structural variation by using genome-wide long mate-pair sequencing in families, which include offspring with severe congenital abnormalities. The resolution for detection of structural variation allows reconstruction of chromosomal rearrangements at nucleotide precision. We identified and characterized simple de novo structural changes as well as complex series of inter- and intra-chromosomal rearrangements consisting of multiple breakpoints involving different chromosomes. Detailed inspection of breakpoint regions for complex rearrangements indicated that in some cases series of simultaneous double-stranded DNA breaks caused local shattering of chromosomes. Fusion of the resulting chromosomal fragments involved non-homologous end-joining since junctions displayed limited or no sequence homology and small insertions and deletions. The pattern of random joining of shattered chromosomal fragments that we observed for some de novo rearrangements, strongly resembles the somatic rearrangement patterns - termed chromothripsis - that have recently been described in deranged cancer cells. We conclude that a similar mechanism may also drive the formation of de novo structural variation in the germline.

P11.096
Four patients with overlapping microdeletions in 9q33.3-q34.1 associated with developmental delay and micro- / brachycephaly

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We report four patients with overlapping microdeletions identified by molecular karyotyping using various array platforms. Their common clinical features include developmental delay with delayed or absent speech and micro- and/or brachycephaly.
In patients 1 through 3, de novo deletions of 1.76 Mb, 1.3 Mb, and 2.8 Mb, respectively, in 9q33.3-q34.11 have been detected. Patient 4 has a maternally inherited deletion of ~432 kb spanning 9q33.3. Her mother is reported to have mild intellectual disability.
The smallest region of overlap of the deletions is defined by the deletion of patient 4 and her mother and includes only three RefSeq genes. Interestingly, the three larger deletions in patients 1, 2, and 3 have 27 genes in common including STXBP1, which is predominantly expressed in brain and plays a role in synaptic transmission. STXBP1 loss-of-function mutations and deletions have been associated with early infantile epileptic encephalopathy (EIEE, or Ohtahara syndrome), and were identified in patients with intellectual disability and non-syndromic epilepsy. Neither patient 1 nor 2 presented with seizures, although an EEG of patient 2 gave pathological results. Thus, the seizure phenotype appears to be incompletely penetrant. Notably, patient 4 presented with epilepsy, although her deletion excludes STXBP1. Thus, the involvement of STXBP1 and/or possibly other genes due to deletion of long range regulatory elements cannot be ruled out.
We suggest that deletions of this region on chromosome 9q may cause epilepsy of incomplete penetrance and a clinical spectrum including developmental delay concerning especially speech, micro- and / or brachycephaly, and mild dysmorphisms.

P11.097
Clinical routine implementation of custom a-CGH in the diagnosis of patients with psychomotor development delay and/or mild-severe mental retardation

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Introduction: The use of array-CGH (comparative genomic hybridization) in clinical routine is already a common practice in many countries. We report the implementation in clinical routine of a custom genome-wide high resolution oligonucleotide aCGH-platform, previously validated (KaryoArray® v3.0), for the study of patients with psychomotor development delay and/or mild to severe mental retardation.
Methods: A prospective study of 100 strictly selected patients with psychomotor development delay and/or deficit cognitive were carried out. Patients with one or more of the following associated findings: structural defects (heart, kidney); facial dysmorphic features; hypo/over-growth disorders; macro/microcephaly; family history of mental retardation. In addition these patients had: karyotype, molecular study of FRAXA and MLPA of subtelomere regions and recurrent-genomic-disorders done previously, with normal results. To end this, we selected 60-mer oligonucleotide features from Agilent's eArray_v4.0 probe library in a custom format of 8x60K (KaryoArray®), covering more than 350 microdeleción/microduplicación syndromes, as well as telomeric/centromeric regions with a resolution from 1 kb in the regions

of interest, with an average density of 7 Kb. The average density of the probe coverage is 43Kb.
Results: we identified in ~30% of these patients at least one genomic imbalance responsible for its phenotype.
Discussion: The implementation of use of KaryoArray® in patients with unexplained development delay/intellectual disability and MCAs offers a much higher diagnostic yield than karyotype and/or currently available platforms for this selected cohort of patients. We highly recommend such kind of aCGH-platform as the first-tier test for individuals with development disabilities or congenital anomalies.
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P11.098
Methylation of mitochondrial D loop in twins

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DNA methylation constitutes an epigenetic DNA modification and plays important cell function through changes in gene expression. In mammalian cells methylation is catalysed by DNA methyltransferase and covalent binding of the methyl group to cytidine is most common. Usually it is the cytosine residue situated 5' in relation to guanosine in CpG dinucleotides that undergoes methylation. Methylation appears also in mitochondrial genome. Due to unique twin genetics, group of twins has been studied as an ideal subject for observations of epigenetic changes. Still, so far no wide range analysis of mtDNA in twins has been reported. In this study the analysis of mitochondrial DNA D-loop in group of newborn twins was performed. Level of DNA methylation in collected samples was estimated using sodium bisulphite assay which leads to a change of 5mC>C and C>U, followed by a PCR reaction and pyrosequencing using PyroQ-CpGT software. Using this approach quantitative analysis was possible and revealed differences in DNA methylation of CpG in mtDNA D-loop. Different levels of methylation were observed within dizygotic and also monozygotic twins pairs and seems to be related to heteroplasmy of mitochondrial genome. In most cases methylation was not observed.
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P11.099
MS-SNuPE as a diagnostic tool for the detection of aberrant methylation patterns in growth retarded patients

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In the last decade the crucial role of imprinted genes in human growth and development has become evident. A number of diseases have been found to be directly linked to aberrant methylation of specific loci including Silver-Russell syndrome (SRS), Beckwith-Wiedemann syndrome (BWS) and Transient Neonatal Diabetes Mellitus (TNDM). A considerable fraction of SRS, BWS and TNDM patients shows aberrant methylation at other than the disease specific loci (multilocus methylation defects - MLMD). To identify epigenetic changes a number of methods have been described. MS-MLPA and MS-PCR as well as MS-pyrosequencing and bisulfite sequencing are meanwhile well established methods. However, they are either time-consuming and/or labor intensive or complex to establish. It is therefore necessary to have simply adjustable assays to analyze different loci with the possibility of direct quantification in single-tube reaction formats available. Based on the AB Prism® SNaPhot technology we established MS-SNuPE (methylation-specific single nucleotide primer extension) approaches to analyze the methylation status of several imprinted loci and to elucidate applicability of MS-SNuPE in research and routine diagnostic. We chose the loci of GRB10, MEST, IGF2R, H19, LIT1, DLK1/GTL2 (chromosomes 6, 7, 11, 14) because methylation of these differentially methylated regions is frequently disturbed in MLMD. The method was validated by screening a cohort of probands with (epi)genetic disturbances identified in previous analyses by other techniques. In all cases, the aberrant methylation was confirmed by the newly developed MS-SNuPE approaches.

P11.100
MicroRNA expression profiling and bioinformatic analysis in human myocardial infarction with focus on innate immunity and ventricular rupture

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Recent studies have indicated the role of microRNAs in various forms of cardiovascular disease, but little is known about the genome-wide microRNAs expression profile in human myocardial infarction (MI). After MI, innate immunity is activated leading to an acute inflammatory reaction. An intensive inflammatory reaction might contribute to the development of ventricular rupture (VR), as well as some microRNAs have influence on the innate immune response. Based on this knowledge, we further analyzed microRNAs in MI patients with and without VR. There were 60 patients with MI and 10 healthy adults. Using microarrays, microRNAs expression was analyzed on human MI compared to healthy adult hearts. Results were validated using qPCR. MicroRNAs target prediction was performed *in silico* (TargetScan, miRanda), and further subjected to functional classification (DAVID). Of 719 microRNAs analyzed, 57 were confidently dysregulated; many of them were not previously related to cardiovascular disease. For 5 microRNAs 37 target genes were predicted, some of them have been already shown to be involved in microRNAs network. qPCR analysis confirmed dysregulation of *miR-150, miR-186, miR-210, miR-451, miR-34a, miR-146a, miR-155, miR-1, miR-133a/b,* and *miR-208*. Comparison of MI patients with VR to those without VR revealed *miR-146a* up-regulation, and *miR-150*/*miR-155* down-regulation. *miR-146a*, *miR-150*, and *miR-155* have been described to play a role in the regulation of the innate immunity. Their differential expression in MI patients with VR compared to those without VR provides further evidence that innate immunity and intense inflammatory reaction play an important role in the VR pathogenesis after MI in humans.

P11.101
Differential expression profile of miRNA in human bloods from preterm infants and adults

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Accumulating evidence suggests a role for microRNAs (miRNAs) in regulating various age-related diseases and human aging. However, little is known about the changes of miRNA expression before and after adulthood. The aim of the study is to investigate whether there are differential expression in miRNAs between infants and adults, and then explore the possible biological functions of these miRNAs. We profiled the expression of 365 miRNAs in peripheral blood mononuclear cells from preterm infants (n=30) and adults (n=60, age ranging from 21 to 60) using real-time RT-PCR analysis. This genome-wide assessment of miRNA expression revealed that the majority of differentially expressed miRNAs increased in adults. Among 365 miRNAs, 228 (62.5%) were detectable in the more than half of the peripheral mononuclear cells in the samples of either adults or preterm-infants. Among these miRNAs, there were 22 miRNAs expressed in the adults' group only, and one (has-miR-325) is specific to the preterm-infants. For the remaining 205 miRNAs, 101 differentially expressed

between the two groups (p<0.00024 with bonferroni correction), in which 81 miRNAs were up-regulated in adults and 20 were down-regulated in preterm-infants samples. Finally, these differentially expressed miRNAs were subjected to the knowledge-base software of ingenuity pathway analyses (IPA, version 8.8) and found that the most significantly associated biological networks were reproductive system disease, cellular assembly and organization, and nervous system development. The results indicated that approximately one third of the miRNAs was modulated from infant to adulthood, and the implication in human development warrants further investigation.

P11.102**

Identification of trait- and disease-relevant genetic polymorphisms in miRNA target sites

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MicroRNAs (miRNAs) are ~22 nucleotide long noncoding RNAs that regulate the expression and function of eukaryotic genomes by acting as adaptors to specifically recognize and degrade targeted mRNAs. Candidate studies suggest that genetic polymorphisms in miRNA target sites (poly-miRTS) are associated with various disorders like Tourette's syndrome, hypertension, or the risk to develop breast cancer. We aim to create a genome-wide catalogue of poly-miRTS to provide the fundamental basis for the characterization of miRNA-related genetic alterations with an impact on human traits and disease, and to unravel the mechanisms of action of some causal poly-miRTS. To identify poly-miRTS in a genome-wide scale, we will monitor RNA levels and differences in allelic expression (AE) with and without active miRNA gene regulation. To shutdown miRNA-mediated gene regulation we interfere with the miRNA processing machinery by silencing Drosha, DGCR8, and/or Exportin-5. Importantly, interference with any of these genes is not expected to prevent siRNA processing. Our present results show that silencing any single gene within the miRNA machinery does not substantially inhibit the level of mature miRNAs. However, successful silencing of Drosha and Exportin-5 leads to a 50% reduction of mature miRNAs. Initial RNA-sequencing and SNP genotyping experiments are underway. To circumvent off-target effects in assessing the global impact of silencing miRNA expression we will monitor genome-wide differences in allelic rather than total gene expression. We anticipate that this effectively eliminates non-specific effects and allows focusing on interaction of miRNA binding and genetic variants at binding sites.

P11.103

Profiling miRNA using semicondutor sequencing technology on the Personal Genome Machine

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RNA-Seq technology has become widely utilized as a tool to understand the transcriptome of a given experimental system. This method utilizes next generation sequencing platforms to sequence a cDNA library in order to gain information about the RNA content and transcriptional status of an experimental system. Profiling the transcriptome of a system in this way has become an invaluable tool in many genomic studies.

The Ion Torrent Personal Genome Machine (PGM™) utilizes revolutionary technology, simplifying next generation sequencing instrumentation in order to make this technique available to a wider group of individuals beyond the basic researcher. The PGM™ instrument combines semiconductor technology with simplified chemistry including natural nucleotides and no enzymatic cascades to create a simple and scalable sequencing platform devoid of the need for expensive and complicated optics and imaging capabilities. This new sequencing platform has successfully provided genomic information for multiple DNA based applications, such as amplicon sequencing, in record time.

Here we report the first RNA-Seq libraries analyzed on the Ion Torrent PGM™ instrument. The miRNA content of multiple RNA types was profiled using novel RNA library preparation reagents. Resulting cDNA libraries were sequenced on the PGM™ in record time providing valuable information on the transcriptome of the interrogated RNAs. This is the first demonstration of the Ion Torrent PGM™ as a simple, fast, and scalable technology for transcriptome analysis using established RNA-Seq library methodology.

P11.104

Plasma mir-155, mir-21 and mir-126 expression profiling in patients with cerebrovascular accident (CVA)

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MicroRNAs (miRNAs) are endogenous 21-25 nucleotides in length RNAs involved in the ethiopathology of various diseases. Recently, plasma miRNAs have been reported to be sensitive and specific biomarkers of various tissue injuries and pathological conditions. miRNAs have distinct expression patterns in stroke etiology, that modulate pathogenic processes including atherosclerosis (*miR-21, miR-126*), hyperlipidemia (*miR-33, miR-125a-5p*), hypertension (*miR-155*), and plaque rupture (*miR-222, miR-210*). The aim of this study was to evaluate some circulating miRNAs expression in plasma patients with cerebrovascular accident (CVA). Blood samples were collected from patients with cardio-vascular disease (CVD) (n=30, aged 57-86 years old, mean = 71.5) who presented hypertension and hyperlipidemia, and from healthy subjects (n=10, aged 20-53 years old, mean = 35.96). 12/30 of the CVD patients presented stroke. Total RNA was extracted from plasma using Trizol reagent. Plasma expression of target miRNAs (*miR-126, 21* and *155*) were assessed by a Real-Time RT-PCR using TaqMan MicroRNA Assays (ABI). Statistical analysis was performed using Mann-Whitney test. qPCR assessment revealed lower plasma levels of *miR-21*, and *miR-126* in CVA group of patients as compared to non-CVA and to the controls. Further evaluation of the miRNA levels in plasma from CVA patients (n =12) demonstrated that *miR-155* levels were substantially lower than those from patients with non-CVA (n = 18, P < 0.03) or healthy people (n = 10, P < 0.01). The results revealed a significantly reduced plasma concentration of *miR-155* in CVA patients indicating it as a potential biomarker for CVA.

P11.105

SM2PH-kb: a novel tool for the integrated study of human missense variants to phenotypes relationships

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Understanding how genetic alterations affect gene products at the molecular level represents a first step in the elucidation of the complex relationships between genotypic and phenotypic variations, and is thus a major challenge in the postgenomic era. SM2PH-kb (http://decrypthon.igbmc.fr/sm2ph) is the second generation of our tool designed to investigate structural and functional impacts of missense variants and their phenotypic effects in the context of human genetic diseases (Friedrich et al Hum Mutat. 2010). We present our recent and future developments together with a practical example (Audo et al Arch Ophthalmol. 2010) on how SM2PH-kb can be used to analyse genotype to phenotype relations.

Up-to-date interconnected information is provided for each of the 2,375 disease-related entries including data retrieved from biological databases (e.g. STRING networks, KEGG pathways, BioGPS tissue gene expression) and data generated from evolutionary inference in biological networks (e.g. multiple alignments, 3D structural models). The 18,700 recorded disease causing mutations have now been complemented by the 143,000 missense SNPs from dbSNP (release 132). The introduction of the Inductive Logic Programming allowed us to automatically, extract knowledge from these data and generate classification rules. This will guide human experts to improve our understanding of the relationships between physico-chemical and evolutionary features and deleterious mutations. This leads to improved prediction of functional effect and pathogenicity of submitted missense variations.

SM2PH-kb provides a robust infrastructure, interactive analysis tools supporting in-depth study and interpretation of the molecular consequences of mutations, with the more long-term goal of elucidating variants pathogenicity.

P11.106
Potentials and pitfalls of Mitochip v.2.0 in the diagnostic of mitochondrial encephalomyopathies

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Background: The identification of mitochondrial disorders due to mutations mtDNA is difficult because the lack of phenotype-genotype correlation and mutation hot spots in the mitochondrial genome. To verify an mtDNA disease the whole mtDNA should have been analyzed.
Methods: Ten patients with neurological and psychiatric symptoms have been investigated. The whole mtDNA was resequenced by MitoChip 2.0 (Affymetrix) according to the manufacturer's instruction. The doubtful SNPs detected by the MitoChip were controlled by bidirectional sequencing with ABI Prism 3100 sequencing machine.
Result: In 1 case a pathogenic heteroplasmic mtDNA substitution A12770G (Glu145Gly) was detected in the ND5 gene. In the other 9 cases many synonym and non-synonym mutations were present .We found 7 unclassified variants in 5 cases. In 2 patients with psychiatric symptoms, axonal neuropathy a unique SNP combination was found which was described previously predisposing for breast cancer and AMD. The haplotype of these patients were: T2, J1c2. These haplogroups harbor substitutions capable of modifying the phenotype of LHON. One of our patients with epilepsy and schizophrenia had haplogroup D4j containing SNPs previously associated with hypoacusis, schizophrenia, LHON. Six of our patients had the typical Hungarian haplogroups (H5, H4, H1, R0, U4). On the base of the MitoChip sequenogram about 22% of the presumed heteroplasmic mtDNA mutations couldn't verified with bidirectional sequencing.
Discussion: We conclude that MitoChip v2.0. is a quick method determining the mtDNA haplotypes and disease modifying homoplasmic SNPs and SNP combinations. To justify the pathogenic heteroplasmic mutations in the mtDNA the bidirectional sequencing is crucial.

P11.107
MUTALYZER 2: Improved Sequence Variant Descriptions from next generation sequencing data and locus-specific mutation databases

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Unambiguous and correct sequence variation descriptions are of utmost importance, not in the least since mistakes and uncertainties may lead to undesired errors in clinical diagnosis. The free Mutalyzer sequence variation nomenclature checker (www.mutalyzer.nl/) names all sequence variants following the Human Genome Variation Society sequence variant nomenclature recommendations (www.hgvs.org/mutnomen), using a GenBank or Locus Region Genomic (LRG) accession number, a HGCN gene symbol and the sequence variant as input. Mutalyzer 2 has new functionality lacking in the commercial Alamut tool used by many DNA diagnostic labs. Mutalyzer generates an output containing a description of the sequence variant at DNA level, the effect on all annotated transcripts, its deduced outcome at protein level and gains or losses of restriction enzyme recognition sites. Mutalyzer facilitates batch-wise conversion from dbSNP rsIDs or chromosomal position numbering used in next generation sequencing data to transcript position numbering, as well as checking of sequence variant descriptions in locus-specific mutation databases (LSDBs). Mutalyzer is also used to quickly check new variant submissions in LSDBs based on LOVD software (www.LOVD.nl/). The new Name Generator can be used to train your self to generate correct HGVS descriptions. New webservices also support the use of Mutalyzer's functionality from other computer programs.
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P11.108
Mutation Hotspot - a web application for mutation mining in publicly available exome sequences

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Genome and exome sequencing has become affordable and, within clinical research projects, it is now reasonable to perform whole genome or exome sequencing for moderately sized cohorts of individuals. Sequence reads from a variety of sequencing technologies are mapped to a reference genome and the analysis of the systematic differences relative to the reference and those shared by different project subjects may be used to identify rare novel gene variants, genes associated with disease or to characterise finer patterns of genomic context.
The assignment of meaning and the identification of patterns within the data are complicated by the large volumes of complex data. To simplify the process of identifying different classes of variation, we have implemented a web-accessible database system for the mining of sequence variation. Starting with primary sequence read data from publicly available exomes, we mapped the reads to a reference human genome. By applying scoring algorithms to the sequence variations characterised within individual exomes, mutations can be filtered on the basis of confidence scores. The total variation has been loaded into a relational database.
Simple user interfaces provide functionality to identify the patterns of variation, namely, those that are common to groups of individuals, those that contrast groups of individuals, and can be used further to identify the polymorphisms that appear unique within individuals. Genes containing variations, genes enriched for multiple variations and genes belonging to functionally related gene sets are linked to external databases so that the meaning and relevance of specific variations can be assessed.

P11.109
Mitochondrial proteins are responsible for Neurodegeneration with Brain Iron Accumulation (NBIA).

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Neurodegeneration with Brain Iron Accumulation (NBIA) comprises a group of neurodegenerative disorders characterized by brain iron deposition and presence of axonal spheroids. Pantothenate Kinase Associated Neurodegeneration (PKAN) and Infantile Neuroaxonal Dystrophy (INAD) are the most frequent forms of the disease and are due to mutations in PANK2, coding for Pantothenate Kinase 2, and PLA2G6, coding for a calcium independent phospholipase. Recently, mutations in an additional gene dubbed FA2H and coding for a Fatty Acid 2-Hydroxylase were found in two families with symptoms resembling NBIA.
Several findings indicate that PANK2 and PLA2G6 are localized into mitochondria, although it is not completely clear in which compartment. In addition, although the putative localization of FA2H was reported to be into the Endoplasmic Reticulum (ER), software prediction for this protein scores high for mitochondrial localization.
Objective of this work is to establish the PANK2 and PLA2G6 sub-mitochondrial localization and to test the hypothesis that the FA2H protein could also be located in this organelle.
We here demonstrated that the three known proteins involved in NBIA are indeed located into mitochondria with the FA2H being present also in the ER. These data indicate that communication between mitochondria and ER is crucial during the transfer of essential lipids and underline the important role of these organelles and lipids metabolism in causing the disease.

P11.110
Parallel copy number variation and sequence variation detection with next generation sequencing (NGS)

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Large genomic duplications and deletions have been recognized as pathogenic mutations for many years. Therefore the determination of gene dosage in combination with the sequence variation detection becomes very important in clinical medicine.
We describe a method for the simultaneous mutation and copy number variation (CNV) detection with next generation sequencing (GS Junior, Roche).
We developed a two-step multiplex PCR for the amplification of all coding exons of 4 candidate genes (RET protooncogen, MEN1, CASR and SHOX) in comparison with 10 control fragments. For validation of the multiplex PCR based method we compared these results with traditional Sanger sequencing and MLPA analysis. One big challange with the multiplex PCR approach is the formation of spurious PCR products due to primer dimerization. For removal of primer dimers or short failed PCR products we used the size-selective solid phase reversible immobilization (SPRI) purification technology. After primer dimer removal, about 95% of all reads are within the specific 300 - 400bp range.
The evaluation of the NGS data as well as the Sanger sequence and MLPA analysis was performed with the Sequence Pilot software (JSI medical systems). For the detection of CNVs, dosage quotients were calculated using control fragments and target fragments for patient and control samples.
All deletions or duplications in the 4 candidate genes, which were known from MLPA analysis, could be detected by NGS. Therefore, the multiplex PCR based method for next generation sequencing is an efficient technique to detect copy number variations and sequence variation simultaneously.

P11.111
Next Generation Sequencing on small amounts of DNA and RNA from FFPE-fixed tissues using NuGEN's Ovation® amplification products

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The analysis of clinical samples has long presented challenges to researchers based on sample availability, amount, and integrity. These challenges are most significant for small and typically degraded formalin-fixed, paraffin embedded (FFPE) samples widely used in cancer research and clinical diagnostics.
We have developed highly sensitive and robust amplification systems for the amplification of DNA and RNA from FFPE tumor samples that take advantage of the proprietary Single Primer Isothermal Amplification (SPIA®) technology developed at NuGEN®.
The whole genome amplification system uses 100 ng of routinely isolated FFPE DNA and produces DNA suitable for multiple downstream applications such as aCGH and next generation sequencing (NGS). Data will be presented that illustrates the performance of the SPIA amplified material benchmarked against microgram quantities of its unamplified counterpart. These studies reveal a preservation of copy number changes and breakpoints.
The whole transcriptome approach used in NuGEN's Ovation RNA-Seq FFPE System, enables amplification and sample preparation of as little as 100 ng of FFPE total RNA for accurate and robust analysis on next generation sequencing (NGS) platforms. RNA isolated from FFPE and Fresh Frozen (FF) matched Colon Tumor and Normal Adjacent Tissue (NAT) pairs was amplified and the products were used to prepare libraries with the NuGEN Encore NGS Multiplex System I and sequenced using the Illumina GAIIx platform.

P11.112
A bioinformatic framework to build junction databases for the detection of alternative splicing isoforms from Next Generation Sequencing data

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The millions of short sequences (reads) generated by next-generation sequencing (NGS) platfroms can be used for a wide range applications including genomewide measurement of gene expression levels (RNAseq), detection of RNA or DNA sequence variations and DNA rearragments.
We were interested in detecting non annotated junctions sequences (JS) at DNA or RNA level. There are several bioinformatic tools (tophat; MMES; gmap; GSNAP) that can do that. However they generally suggest a huge amount JSs that have to be verified. A first validation step can be conducted in silico generating all the sequences corresponding to a JS. If several reads map on a JS, then we have a first indication that the JS suggested by the bioinformatic tools is likely to be a true JS. Therefore we developed a computer program able to generate all possible JSs using genomic coordinates (chromosome name, start and stop positions) of a reference sequence. This tool has now been used to create in silico all possible annotated isoforms for a given gene to check if some unmapped short reads might map on JSs of a specific isoform. This tool can be also used to get an individual sequence from a genome reference sequence by indicating the genomic coordinates of the multiple fragments of interest. The program is included in the Short Reads ToolKit (http://ddlab.sci.univr.it/srtk/), a Python library of functions and programs dedicated to NGS data computation.

P11.113
Highly multiplexed barcoded enrichment of up to 96 samples for next-generation sequencing

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The unprecedented increase in throughput of next-generation sequencing (NGS) technologies allows for efficient sequencing of multiple exon-centric samples in a single sequencing run. However, library preparation and enrichment of multiple samples has now become a limiting step for high-throughput genomic analysis. To address this, we developed an efficient strategy for enriching pools of pre-barcoded samples using both microarray-based as well as solution-based approaches. We have optimized custom protocols and pipelines for probe design, library preparation, enrichment and variant analysis and prioritization. The multiplexed enrichment protocol is highly efficient and provides the experimental flexibility that meets the needs of various types of variant discovery projects, ranging from small families to population studies, while screening small sets of candidate genes or large genomic intervals. We found that performance for pools between 3 and up to 96 samples is similar in comparison to non-multiplexed samples.
Here, we focus on detection of causal variants using X-chromosome exome sequencing in families with an X-linked inheritance pattern. We designed a custom enrichment array for all protein and RNA coding genes on human chromosome X with a densely tiled probe overlap, which resulted in a very even coverage distribution (>99% of all positions covered by at least 1 read). Using this array, it is possible to enrich approximately 30 individuals from different families in a single assay, which fits the capacity of a single SOLiD sequencing run and provides a flexible strategy for high-throughput variant detection with significantly lowered costs and efforts.

P11.114
Development of an exome sequencing workflow in a diagnostic setting

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For many years Sanger sequencing has been the golden standard and workhorse in DNA diagnostic laboratories. However, implementing gene tests for all known monogenic diseases is impossible in single laboratories. Moreover, the cause of many genetic diseases is still

unknown. Together with genetic heterogeneity adding another level of complexity to clinical diagnosis, diagnostic yield remains overall relatively low. Targeted Next Generation Sequencing approaches allows rapid and affordable analysis of genetic variation in multiple loci in parallel. This has been of enormous value in recent disease gene identification studies, especially when expanded to the exome. In a recent family-based exome sequencing study we showed that rare *de novo* mutations can be reliably identified and may explain the majority of cases with mental retardation (Vissers et al, 2010). These results demonstrate that exome sequencing is becoming a robust approach for identification of genetic variation and can be implemented in a diagnostic setting, even for genetically heterogeneous and largely unexplained common disorders like mental retardation.
In this presentation we will describe the development and validation of a diagnostic workflow for exome sequencing in genetically heterogeneous disorders such as hereditary blindness, hereditary deafness, mitochondrial diseases, movement disorders and mental retardation. Important aspects of the implementation of NGS as a diagnostic tool will be addressed; the informed consent procedure, description into our quality system, the laboratory and data-analysis workflow, as well as the reporting to referring clinicians. This will be followed by a discussion on the results of our first diagnostic exome sequencing analyses.

P11.115
Exome sequencing of an italian patient with dHMN

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Distal hereditary motor neuronopathy (dHMN) is heterogeneous group of disorders characterized by an exclusive involvement of the motor part of the peripheral nervous system. Often, unusual or additional features are present in 'complicated' distal HMN, including predominance in the hands, vocal cord paralysis, diaphragm paralysis and pyramidal tract signs. To date 16 loci and height genes have been identified. We have previously mapped a novel locus in an Italian family with dHMN complicated by pyramidal signs on chromosome 4q35, in a 26cM region between markers D4S1552 and D4S2930.
The full exome of the proband from the family was submitted to a capturing/enrichment process with SureSelect target Enrichment system (Illumina/Solexa) technology. The captured DNA was submitted to a high throughput sequencing using the Genome Analyzer II (Illumina). We were able to obtain 0.9 Gb of genomics sequences from two runs. The raw data was trimmed and aligned with the human genome reference (hg19) using MAQ software. We identified 65508 high-confidence single nucleotide variants and 87 deletion/insertion polymorphisms (DIPs). After the reads stringencies and based on linkage evidences, the results showed 80 unique variants that are not present in dbSNP (v.131) and 2 novel synonymous DIPs in heterozygous state. The identified variation was confirmed by Sanger sequencing and will be assessed in all members of the family and in a normal control population.
Exome sequencing of a proband from the families with linkage evidence is a powerful and efficient strategy for identifying the genes underlying rare mendelian disorders.

P11.116
Novel sequencing primer and simplified PCR-Sanger sequencing workflow increase 5' resolution and throughput

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High quality and high accuracy are the hallmarks of Sanger re-sequencing projects. We have developed new sequencing primers and workflow that improve 5' sequence resolution, increase throughput, and reduce hands-on time. The novel sequencing primer chemistry and workflow produces high quality sequence data from base 1 on POP-7™ polymer that previously only could be resolved with longer run time on POP-6™ polymer.
The new primer chemistry and workflow, BigDye® Direct Cycle Sequencing, eliminates the need for a separate PCR clean-up step, thereby saving time and cost and reducing possible pipetting errors. These improvements reduce the entire workflow time for PCR to finished sequence data to less than 5 hours, compared to 8 hours for the standard workflow.
We have sequenced many exon sequences, including challenging templates, using our enhanced sequencing primers and workflow on an Applied Biosystems 3500*xl* Genetic Analyzer. We compared 5' resolution, basecalling accuracy and quality of electropherograms generated with traditional sequencing primers and workflows versus BigDye® Direct novel primers and workflow.
On average, the new primers produced high quality bases by base 5, and by base 1 in many cases, compared with base 25 with traditional primers on POP-7™ polymer. The superior resolution increased basecalling accuracy, both in the 5' end and in mixed base positions. In conclusion, the simplified BigDye® Direct Cycle Sequencing workflow generates data superior in quality relative to other currently used reagents and offers significant time savings up to 40%. The product is for research use only. Not for use in diagnostic procedures.

P11.117
Comparison of solution based exome capture methods for next generation sequencing

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Techniques enabling targeted re-sequencing of the best-understood regions - the protein coding sequences - of the human genome on next generation sequencing instruments are of great interest. We conducted a systematic comparison of the solution based exome capture kits provided by Agilent (SureSelect and SureSelect 50Mb) and Roche NimbleGen (SeqCap and SeqCap v2.0), to weigh their advantages and disadvantages.
A control DNA sample was captured with all four capture methods and prepared for Illumina GAII sequencing. Protocols provided by the manufacturers were slightly modified for equalization. Read counts were normalized to comparable amounts of high quality, non-duplicated reads. Sequence data from additional samples (n 28) prepared with the same protocols in our laboratory for other projects were used in the analyses. For the data analysis a bioinformatics pipeline for QC, variant identification and annotation (VCP) was also developed. The performance of the capture methods was evaluated for sensitivity and specificity of the enrichment of the target regions, ability to identify known and novel variants, and correlation of sequenced variants and genotypes from Illumina 660K Chip.
Both the target designs and the actual captured regions varied between the capture methods. While Agilent kits targeted more regions, sequences from the NimbleGen capture covered more CCDS exons and genes with a minimum of 20x coverage. There was virtually no difference in the concordance compared to chip genotypes, and a minimum of 11x coverage was required to make a heterozygote genotype call with 99% accuracy when compared to common SNPs on GWA arrays.

P11.118
Exact Call Chemistry enables superior accuracy in next-generation sequencing

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Exact Call Chemistry (ECC) is a unique ligation-based sequencing methodology that builds on the SOLiD™ system chemistry. While every base of the DNA template is interrogated twice using two-base encoded probes, ECC performs an additional inspection of the template using a new, four-base encoded probe set that is carefully designed to complement two-base encoding and jointly form a redundant error-correction code. This strategy allows for error detection and correction without the use of a reference sequence. We demonstrate the performance of ECC in the sequencing of human genomes and compare the performance to that of two-base encoding in the absence of ECC. ECC increases overall throughput including over 60% more reads with zero mismatches compared to the reference sequence. We use ECC to detect SNPs and structural variations in these human genomes and demonstrate that the more accurate reads translate to improved variant detection. Using human genomes with 10-14x coverage, we detect 5% more SNPs with ECC and 30% to 100% more small indels (deletions up to size 11 and insertions up to size 3) without loss of specificity using a split-read approach. ECC chemistry also enables the optimization of sample preparation techniques to reduce errors in these steps. Additionally, we introduce reference-assisted ECC sequencing which further increases accuracy. The multi-base encoding functionality of ECC contributes to a lower error rate and reduced systematic noise, resulting in unsurpassed sequencing accuracy and variant discovery.

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P11.119

A New Sequencing Workflow Increases Accuracy and 5' Resolution for Capillary Electrophoresis Resequencing Applications

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Applied Biosystems™ introduces the BigDye® Direct Cycle Sequencing Kit: BigDye® terminator chemistry that combines post PCR clean up with cycle sequencing steps for increased throughput. BigDye® Direct (BDD) uses M13 universal primers and is ideal for resequencing applications. BigDye® Direct improves 5' sequence resolution through novel sequencing primer chemistry producing high quality bases from base 1 on POP-7™ polymer that previously only could be resolved with longer run time POP-6™. Some of the benefits that BigDye® Direct Cycle Sequencing Kit provides include no sample transfer between PCR and sequencing reactions, the complete workflow is performed in the same well of a plate, thus saving time and labor and reducing human error. In conjunction with the BigDye® Direct Cycle Sequencing Kit are new mobility files for improved basecalling accuracy. Here we describe BigDye® Direct sequence reads that are equal to or better than BigDye® Terminator v3.1 reads when the reactions are analyzed using POP-7™. Further, BigDye® Direct is able to achieve readable bases after the sequencing primers and high quality bases after the gene-specific primer sequences. Nearly 40% of BigDye® Direct reads have ≥25 bases with QV20+ scores when compared to the same amplicons sequenced using BigDye® Terminator v3.1. BigDye® Direct can resolve peak compression problems that occur when certain amplicons are sequenced with BigDye® Terminator v1.1 chemistry. In conclusion, the novel primer chemistry and workflow generates data superior in quality when compared to current chemistry solutions and obtains readable sequence within 5 bases of the primer offering improved polymorphism detection.

P11.120***

Variant calling experience from 150 human exome sequences

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Exome sequencing provides an approach to detect causative mutations, especially in a clinical context where clinical and familiar information can assist an informed choice of candidate SNPs. We first analyzed 11 exomes to compare:

a) Alignment algorithms: Maq, BWA and Novoalign,

b) SNP and Indel identification: Maq, Samtools and Dindel

This allowed us to compare the results obtained using different combinations of aligners/variant callers. 11,596±631 described and 280±88 novel SNPs and 59±3 described and 10±4 novel Indels were called by all the methods. The comparison lead us to Novoalign as our aligner of choice, Samtools to call the SNPs and Dindel for Indel calling.

With those, we processed 140 exomes, analyzing effects, frequency and distribution of the variants identified and comparing them with the characteristics of the variants in ENSEMBL and the 1000 genomes consortium. This analysis allowed us to characterize Indels across a sizeable dataset. None of our calls exceeds 15bp length, most of them being smaller than 6bp. The correlation of the size of the Indel identified with its described counterpart in Ensembl is 0.7642 for insertions and 0.6118 for deletions. Most of the described variations fall within 10bp from the starting point of the Indel called in our dataset. Frameshift coding consequences represent 49.56% of all the indels annotated by ENSEMBL, while being 5.97% of the known Indels and 27.89% of the novel ones in our data. Non synonymous-coding indels represent 2.59% in ENSEMBL Indels, 9.13% of our described calls and 5.47% of our novel ones.

P11.121

Simultaneous Evaluation of Small RNA, Whole Transcriptome, Whole Genome and Targeted Resequencing on Next Generation Sequencing Platforms

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Researchers are using Next Generation Sequencing platforms to support more and more applications. It is critical for such platforms to be flexible enough to support many applications at the same time while maintaining low cost, high quality and throughput. In this study we describe the development of a next generation sequencing process that facilitates the interrogation of up to twelve independent samples or applications run simultaneously. In a single run we sequenced a single-plex human whole transcriptome sample, a multi-plex of universal human reference, human placenta, human brain reference and HeLa whole transcriptome sample, a microbial whole genome sample, and a human placenta smRNA sample. Each sample type used required a different sequencing run, yet with this new process they all were run together saving time, reagents, and freeing the researcher from having to wait to collect sample of the same type to run together.

P11.122

An Easy to Use Workflow for Next Generation Sequencing

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A flexible and easy to use workflow is critical for any next generation sequencing platform. As part of such workflow the 5500 and 5500xl SOLiD™ Sequencers are the latest next generation sequencing systems to be introduced by Life Technologies. Both systems fit on a bench top and are designed to be user-friendly from a hardware, consumable and software point of view. Improvements to the system are designed to support the list of ever expanding applications on next generation sequencing platforms with fast time to result, scalable throughput, low run and acquisition cost, and accurate data. Here we describe fluidic, optic, stage, and electronic innovations to reduce cycling, imaging, and processing times. New reagent configurations allow for more flexibility in desired read length and high accuracy output. Other novel features include an internal control that provides real time feedback on the quality of the run and microfluidic chambers that allow interrogation of up to twelve independent samples or applications at a time, such as whole genome, targeted resequencing, small RNA and whole transcriptome. New software concepts include an intuitive user interface for run setup, sequence quality monitoring, primary analysis, and troubleshooting. For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

P11.123
Semiconductor Sequencing for Life

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Ion Torrent has invented the first device_a new semiconductor chip_ capable of directly translating chemical signals into digital information. The first application of this technology is sequencing DNA. The device leverages decades of semiconductor technology advances, and in just a few years has brought the entire design, fabrication and supply chain infrastructure of that industry_a trillion dollar investment_to bear on the challenge of sequencing. All of these benefits are a result of applying a technology that is massively scalable, as proven by Moore's Law, to a task that has traditionally used optics-based solutions, which work in a linear fashion: increasing capacity requires increasing the number of signals that must be read resulting in longer run times, higher capital costs and ever more sophisticated optics. By contrast, Ion Torrent semiconductor technology can provide increases in chip capacity without impacting capital costs or runtime. Ion Torrent sequencing uses only natural (label-free) reagents and takes place in Ion semiconductor microchips that contain sensors which have been fabricated as individual electronic detectors, allowing one sequence read per sensor. We will show how the technology has scaled in just a few months from ~1 million sensors in the first-generation Ion 314 chips to ~7 million sensors in the second-generation Ion 316 chips_all while maintaining the same 1- to 2-hour runtime. We will also demonstrate that Ion semiconductor sequencing provides exceptional accuracy, long read length and scalability on a single, affordable bench-top sequencing platform.

P11.124
Whole Genome sequencing in patients with the Hallermann-Streiff Syndrome

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The recent introduction of next-generation sequencing has shifted the focus of gene identification in human diseases from Sanger sequencing and copy number analysis to deep sequencing of linkage intervals, targeted regions and exomes. Although the sequencing technologies provide solid data the main bottleneck remains the isolation and enrichment of these specific regions. Sequence capture on arrays or in solution has significantly improved over the last years but introduces a bias in sequence recovery and still misses a significant proportion of the targeted regions. Deep sequencing of whole human genomes has now become available through the company Complete Genomics. The coverage exceeds 40X for 95% of the exome while repeat-free intra- and intergenic regions are well covered too. The overall error rate is estimated at 1 in 100,000 nucleotides. Through their service, we have sequenced the genomes of three patients with the Hallermann-Streiff syndrome (HSS) as well as the parents of one of these patients. Since the inheritance pattern of HSS strongly points to an autosomal dominant disorder, we aimed to search for de novo variants in genes based on the trios samples, and then look for unknown variants in overlapping genes in the other two patients. For the five samples we obtained 160 to 250 Gb of mapped data, which contained about 3.2 million SNPs each. We are currently validating a set of candidate variations in our patients. We will present data and discuss the experimental set-up in order to maximize the screening efficiency.

P11.125
The immense genomic variability of human olfactory receptor genes

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Humans are highly variable in their olfactory sensitivity and odor quality perception. This is partially attributed to genetic variations in the olfactory receptor (OR) coding regions. Specifically deleterious variations, such as frame-disrupting single nucleotide polymorphism (SNP) or copy number variation (CNV) with deletion alleles, are excellent candidates to underlie this phenomenon. One example is a significant association which we have discovered between nonsense SNP in OR11H7P and sensitivity for isovaleric acid. To provide a genomic infrastructure for associating OR genotypes to olfactory phenotypes we applied in-silico data mining, combined with Next Generation Sequencing (NGS) to the OR sub-genome and transcriptome sequences. Scrutinizing dbSNP, the 1000 Genomes Project (1000GP), and several published individual genomes, we identified 285 deleterious variations in 210 ORs. Using the recently published CopySeq algorithm, applied to the low-coverage 1000GP data we obtained 72 OR genes with deletion allele. Altogether, we find that ~60% of the OR genes segregate in the human population between an intact and disrupted allele, indicating huge functional variability and suggesting that each human on the planet has a different nose. NGS transcriptome analysis of human olfactory epithelium unravels novel mutation targets, including 412 genes that are X10 overexpressed compared to control tissues. In addition, we successfully assembled from the data 452 novel transcripts that are in the OR gene territories uncovering yet uncharacterized untranslated exons in ORs and in olfactory transduction genes (targets for general olfactory sensitivity phenotypes). Many of these variations are presently tested for genetic association with odorant threshold phenotypes.

P11.126
Characterization of CD4+ NKG2D+ T lymphocytes based on whole genome expression analysis

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The NKG2D activating receptor is expressed on γδ T, αβ CD8+ T, NKT and all NK cells but not on CD4+ T cells. However, under certain circumstances, such as infections, autoimmune diseases or tumors, an anomalous expression of NKG2D on CD4+ T cells can be detected. Flow cytometric analysis of NKG2D expression on CD4+ T lymphocytes from several transplanted patients (kidney, heart, liver) and healthy donors revealed that kidney and liver transplanted patients presented an unusual subset of CD4+ NKG2D+ T lymphocytes (41.0% and 27.6% respectively) whereas only in a negligible number of heart transplanted and control donors this cell type could be detected.
Whole genome microarray expression studies were performed with this CD4+ NKG2D+ T cell population compared to CD4+ NKG2D- T cells in order to determinate its phenotypic and functional characteristics. Furthermore, functional analyses using Gene Ontology annotations were conducted in order to identify molecular pathways that exhibit activation or repression in this cell type.
Functional analysis showed that several pathways associated with immune response, apoptosis, inflammatory response and cellular catabolic processes were significantly affected. Examination of costimulatory molecules and specific cytotoxic cell markers as well as apoptosis-related molecules showed that the CD4+ NKG2D+ T cells in the kidney and liver transplanted patients represented a highly differentiated T cell subset with cytotoxicity ability and apoptosis resistance. The identification of biomarkers to predict the allograft outcome and immune response in transplanted patients is decisive to decrease the current transplantation failure.

P11.127
Non-coding elements of disease-causing genes

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Three percent of the human genome consists of conserved non-coding elements (CNEs). Those elements are non-transcribed or transcribed such as microRNAs. In the present work, we considered three groups of genetic diseases: diseases with mapped loci but no known genes (group I), diseases with cloned genes (group II), and diseases with recurrent chromosomal rearrangement (group III). To address the interest of studying non-coding elements in disease-causing genes for which mutations are already known and characterized, we plotted group II together with the location of microRNAs. Interestingly, we

could identify 274 diseases with cloned genes that may still involve microRNAs. In particular, we were able to identify a notable enrichment in monogenic diseases with clinical heterogeneity in this category. By comparative genomics alignments across multiple vertebrate species we then selected possibly functional CNEs. We further aimed at establishing functional linkages between these CNEs and their likely target gene by replacing them in an ancestral mammalian genome, and postulating the maintenance of CNE/target gene physical proximity in ancestral and modern genomes. This approach led us to the identification of 72 disease-causing genes putatively controlled by CNEs and involved in diseases that are listed herein. Our results provide a source of non-coding elements associated with disease-causing genes to be sequenced in those patients with no mutation previously identified. It also paves the way to unravel sets of CNEs aiming to the concerted phenotypic-specific regulation of a disease-causing gene, otherwise involved in numerous developmental fields.

P11.128

Disruption of the histone acetyltransferase MYST4 leads to a Noonan syndrome-like phenotype and hyperactivated MAPK signaling

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Epigenetic regulation of gene expression through covalent modification of histones is a key process controlling growth and development. Breakpoint mapping of a balanced *de novo* chromosomal translocation t(10;13)(q22.3;q34) in an individual with a Noonan syndrome-like phenotype encompassing short stature, blepharoptosis and attention deficit/hyperactivity disorder, identified haploinsufficiency of the MYST4 gene. MYST4 belongs to the family of MYST histone acetyltransferases (HAT) and specifically acetylates H3k14. We observed a correlation of MYST4 levels with H3 acetylation in two cell lines after siRNA knock down (Rank Correlation coefficient p < 0.02793). In Mice homozygous for a hypomorphic gene trap allele of *Myst4* [*Querkopf* (*Qkf*$^{gt/gt}$)] we found a failure to thrive phenotype and high expression in the primordial of the cartilage and the neuronal stem cells. Genome wide transcriptome profiles in three human and two mice cell lines uncovered an enrichment of significantly differentially expressed genes in 10 KEGG pathways. To distinguish between primary and secondary targets we performed ChIP-CHIP and found enrichment only for the MAPK signaling pathway. As hyperactivation of the RAS-MAPK transduction pathway is the main underlying pathomechanism in Noonan syndrome we confirmed a hyperactivation of the MAPK pathway by hyperphosphorylation of key proteins by MYST4. In addition, we were able to reverse this effect by MYST4 overexpression in MYST4 deficient cell lines.
This further elucidates the complex role of histone modifications in mammalian development and adds a new pathomechanism to the phenotypes resulting from misregulation of the RAS signaling pathway.

P11.129

Methylation markers of early-stage non-small cell lung cancer

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DNA methylation changes are common and relatively stable in various types of cancer and may be used as diagnostic or prognostic biomarkers. To gain an insight into the epigenetic dysregulation in NSCLC, we've analysed stage I NSCLC samples from 48 patients together with 18 matching macroscopically cancer-free control samples with Infinium™ HumanMethylation27 BeadChips (Illumina Inc., San Diego, CA, USA) that cover the predicted promoter regions of over 14 500 genes.
Cluster analysis was performed with Limma program of Bioconductor package in R statistical computing software. We've detected 496 CpG-s in 379 genes hyper- and 373 CpG-s in 335 genes and pseudogenes hypomethylated in NSCLC. All the methylation differences were identified with FDR corrected p<0.05 and ≥0.136 mean difference in methylation level. Analysis with Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, Redwood City, CA, USA) showed that the top functions of these genes were cell-to-cell signaling and interaction, hematological system development and function, immune cell trafficking, cellular growth and proliferation, cell death, cancer, inflammatory response etc. The most prominently represented gene network was related to tumor necrosis factor (TNF). Comparing methylation data with gene expression data, we found form the hypermethylated genes 41 downregulated and from the hypomethylated genes 12 upregulated. Many of these hypermethylated and downregulated genes haven't been reported yet to be involved with lung cancer but some of them (RASSF1, AXUD1) are known or proposed to be tumor suppressors in lung cancer progression. From the hypomethylated and upregulated genes the most interesting finding was SERPINB5 (Maspin).

P11.130

Distal gene regulatory elements have an average size of a nucleosome

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Nucleosomes consists of 147 bp of DNA wrapped around eight histone proteins, with a normal distance of 20-30 bp between the nucleosome ends. Transcription factors (TFs) bind to promoters and distal regulatory elements and regulate the activity of nearby genes. Promoters located upstream of genes are well known but distal regulatory elements are poorly defined as to location and size. Using 200 million aligned reads from MNase I digested HepG2 chromatin, we created genome-wide nucleosome profiles. These were correlated to ChIP-seq results for the TFs HNF4a and FOXA2, which bind to distal regulatory elements. The patterns suggest that one nucleosome is displaced at the regulatory elements. Footprints of nucleosomes around public ENCODE data of DNase I hypersensitivity sites at distal locations also suggests that in general, a single nucleosome is removed by the TF complexes.
To apply the strategy to a gene of medical interest, we studied SNPs identified in GWAS studies for dose requirement of the drug warfarin, which is commonly used to prevent thrombosis. These studies have identified several SNPs in high linkage disequilibrium in and around the *VKORC1* gene, but the functional one has not been identified. We used several chromatin signals and FAIRE DNA as a measure of open chromatin to prioritize among *VKORC1* SNPs and found that most of them are located in chromatin without regulatory potential. Out of 22 tested *VKORC1* SNPs, 7 are located in nucleosome-free regions as determined by MNase-seq and qPCR of FAIRE DNA, which indicates that they may be functional.

P11.131

Distillation of potential drug targets for type 2 diabetes by overlaying TCF7L2 ChIP-seq and pancreatic islet open chromatin maps

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Variation in TCF7L2 is strongly associated with type 2 diabetes (T2D). We previously mapped the genomic regions bound by TCF7L2 using ChIP-seq. The list of genes bound by TCF7L2 harbor a highly significant over-representation of loci uncovered in GWAS of T2D. Further support for our conclusions came from a subsequent VDR ChIP-seq study that gave a similar enrichment of GWAS-discovered genes. Therefore, it is our hypothesis that a substantial portion of the reminder of genes bound by TCF7L2 on our list, not previously implicated in disease, also harbor genetic variants associated with T2D. We aimed at distilling down genes bound by TCF7L2 that encode

proteins that would be considered attractive drug targets. We analyzed the overlap of loci between our TCF7L2 ChiP-seq gene list and the published open chromatin map for pancreatic islets in order to get an indirect measure of relevant sites in this key tissue. We found that 94 genes overlapped, and interestingly, 36% of the gene products fall in to drug target classes. Attempting to distill this gene list further, we also carried out ChIP-seq in HepG2 cells where TCF7L2 is abundantly expressed. 24 genes remained of which 7 encode what would be considered druggable targets. In summary, we have distilled down loci that are both bound by TCF7L2 across two cell lines and coincide with open chromatin in human pancreatic islets, of which a number encode potentially attractive drug targets that are also likely candidates to be genetically validated when sequenced in a T2D cohort.

P11.132
Identifying Candidate Genes for Phenotypes in CNV Diseases by Computational Model Organism Comparisons

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An accurate description of phenotypic abnormalities is the foundation of clinical diagnostics and the basis of our understanding of diseases. The Human Phenotype Ontology (HPO) was developed by our group as a hierarchical, standardized vocabulary for human phenotypes in human monogenic and other diseases (http://www.human-phenotype-ontology.org).

Applying standardized vocabularies is the basis of a computational interspecies comparison of phenotypic features and disease models. Using phenotypic data for human CNV diseases (HPO), as well as zebrafish (ZFIN, Oregon) and mouse phenotypes (MGI, Jackson Laboratory, Cambridge and Bar Harbor), we have developed novel strategies for computational interspecies comparisons and applied these phenotypic similarity algorithms to identify the disease causing genes in recurrent human copy number variation disorders (CNV disorders). We examined the relationships between phenotypes associated with CNV disorders and phenotypes associated with humans or model organisms with single-gene diseases whose genes are located within the CNVs in order to identify previously unrecognized genotype-phenotype associations by using interspecies phenotypic comparisons to uncover the pathogenesis of CNV disorders.

Our method reliably detected known associations such as the relationship between elastin haploinsufficiency and supravalvular aortic stenosis in Williams syndrome as well as a host of novel explanations for phenotypic features of CNV disorders including candidate genes for cancer risk in CNV-related Sotos syndrome and for diabetes mellitus in Williams syndrome. Our methods for interspecies comparison will provide a basis for integrating the large amounts of model organism phenotype data expected to come from the international knockout mouse consortium and other projects.

P11.133
EnSpm-N6_DR DNA transposons shape the repertoire of p53 target genes in zebrafish

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Transposable elements (TEs) represent a considerable fraction of the genome of eukaryotes. With their high turnover, TEs play a strong role in genomes evolution and adaptation. They directly regulate the expression of nearby genes both transcriptionally and post-transcriptionally. For instance, TEs are known to donate a wide variety of gene regulatory sequences to the human genome, which exert diversifying effects on the expression patterns of adjacent genes. Here we report their role in shaping the repertoire of a transcription factor, the pleiotropic master regulator p53. The multiple copies of En-SpmN6_DR non-autonomous DNA transposon are mapping throughout the genome of zebrafish (*Danio rerio*), mainly upstream or in the introns (less frequently just downstream) of genes, like *trim8.1*, whose human orthologs are annotated as putative p53 targets. We thus assessed if the p53 transcription regulator was able to bind members of this family of TEs, through a putative p53 responsive elements (RE) mapping within the EnSpm-N6_DR transposons. By luciferase assay, we showed that this well conserved p53 binding site can be directly associated with p53-dependent transcriptional activation of multiple zebrafish orthologs, including *trim8.1* and *spred2*. Our data strongly support the hypothesis that the EnSpm-N6_DR class of mobile genetic element promoted the spreading of p53RE within *Danio rerio*'s genome, potentially illustrating a remarkable case of convergent evolution.

P11.134
A new missense mutation (p.L481R) of the ABCB4 gene segregates in three affected members belonging to one large family with more siblings affected by recurrent cholelithiasis and/or cholestatic diseases

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Progressive intrahepatic cholestasis type 3 associated with complete absence of biliary phosphatidylcholine (PC) results in liver failure at pediatric age.

This phenotype is caused by 2 ABCB4 alleles which encode a protein without floppase activity across the canalicular membrane of the hepatocyte.

Liver failure associated with absence of biliary PC was originally described in mice knockout for the orthologous ABCB4 gene whereas no detectable pathology was then reported in mice with a single mutant allele.

Molecular analysis of the ABCB4 by sequencing the 27 coding exons was carried out in three siblings, a 45 year old man and two women, 41 and 47 years old.

All had experienced recurring symptomatic cholelithiasis, the younger sister has also presented an episode of cholangitis and pruritus in pregnancy.

In these siblings we identified the new mutation c.1442T>G (absent in 110 controls), in the heterozygous state, which leads to an amino acid change (p.L481R) in the Nucleotide Binding Domain; the family history suggests paternal lineage of the mutation: their father with a history of cholelithiasis died at the age of 56 years with biliary cirrhosis.

This family includes others 12 siblings, 4 with symptomatic cholelithiasis (their DNA is not currently available). Although it is plausible that additional genetic loci (and environmental factors) with modifier effect may be involved, the analysis of segregation in 3 siblings suggests that a single mutant allele of the ABCB4 can be a critical factor for susceptibility to the manifestation of cholelithiasis or progressive liver disease in adulthood.

P11.135
Multi-omics Landscape of Clear Cell Renal Carcinoma

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Tumor is considered to be a systematic disorder of molecular network in tissue cells, while the incidence of renal cell carcinoma (RCC) has a significant and continually increasing trend. In our study, we present a comprehensive (more than 250 Gbp) data set from whole genome, DNA methylome and transcriptome sequencing of RCC. By comparison between RCC and corresponding para-carcinoma (PARA) tissues, we identified 43428 single nucleotide variations (SNVs), with 168 in coding region, 5448 insertions and deletions (indels, 1-10 bp)

and 9095 segmental rearrangements or copy number changes (SVs/CNVs, 1k-50k bp). We discovered 24187 differentially methylated regions and 8416 differentially expressed protein-coding genes as well as non-coding RNAs between RCC and PARA. We attempted to integrate all information from these omics levels to detect the inter-correlations in-between, and the discovered patterns are associated with Focal adhesion, MAPK signaling and other pathways related to cell metabolism, proliferation and development. The methylation pattern and expression level indicates IFI16 gene, which encoding interferon, gamma-inducible protein 16, not only modulates p53 function, but also plays an important role, as a hub gene, in apoptosis pathway. Our results demonstrate an initial investigation of whole omics landscape of tumor tissues and pave a new way to understanding the important disease by multi-omics sequencing approaches.

P11.136

Novel study of copy number variations in EYS using the multiple ligation-dependent probe amplification (MLPA) technique

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Retinitis Pigmentosa (RP) is a heterogeneous group of inherited retinal dystrophies characterised by the loss of photoreceptors cells and retinal pigment deposits. Autosomal-recessive Retinitis Pigmentosa (arRP) has recently been associated with mutations in a novel gene, EYS, which is a major gene for this disease. All published mutations so far are based upon conventional PCR and are not adequate to identify mid-sized DNA rearrangements. The purpose of this study was to establish the prevalence of Copy-Number Variations (CNVs) in the EYS gene, using a novel EYS specific MLPA kit, in a cohort of arRP patients, including individuals in whom only one pathogenic change was detected by PCR-based sequencing. Here we report the identification of 6 pathogenic CNVs and confirm the presence of mid-sized rearrangements as the second mutated allele in a ~15% of families that were carriers of another EYS mutation. Our results also suggest that mid-sized genomic rearrangements in EYS gene would be a common event in the appearance of RP phenotype with an estimated frequency of ~4% (3/71) of total arRP cases with unknown mutation. We have demonstrated an efficient and cost-effective strategy validating a novel MLPA Kit as a complementary diagnostic method for EYS pathogenic evaluation.

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Haplotype analysis of *EYS* mutations identified in different European populations

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Retinitis Pigmentosa (RP) is a heterogeneous group of inherited disorders primarily affecting rods with secondary cone degeneration. It is the most common inherited form of potentially severe retinal degeneration and blindness, with a frequency of about 1 in 4000 births. Inheritance of RP can be either autosomal recessive, autosomal dominant or X chromosomal with also rare cases of mitochondrial transmission. Recessive and isolated cases account for 50-60% of all forms of RP. Recently, we have identified a new gene, *EYS,* corresponding to the *RP25* locus, as a commonly mutated gene in arRP. Spanning over 2 Mb within *RP25*, *EYS* is the largest eye-specific gene identified so far. Population studies in cohorts of different origins have been performed, resulting in the identification of more than 70 mutations so far. Among them, 3 pathogenic variants have been recurrently reported in Spanish and Dutch populations: p.I2239SfsX17, p.W2640X and p.Y3135X. In this study, we performed the haplotype analysis in the non related families bearing those mutations with the aim of elucidating their recurrent or founder origin. For that purpose, we used both microsatellite and SNPs genotyping covering *EYS* and the specific mutations regions, respectively. *EYS* flanking microsatellite haplotypes were different among families in all the mutation groups, suggesting a recurrent origin of the variants. However, SNPs genotyping of the mutation intervals allowed a higher resolution haplotyping. According to these data, we can confirm that mutations p.W2640X and p.Y3135X have an ancestral origin, each of them transmitted by a common founder, whereas p.I2239SfsX17 is recurrent.

P11.138**

Targeted next generation sequencing as a powerful diagnostic tool for RP

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Next generation sequencing (NGS) opens the way to high throughput analysis of targeted genomic regions or even the whole exome/genome. Recent publications show the advantages of NGS in genetic research, e.g. in gene discovery in rare inherited syndromes. However, for the usage of NGS in diagnostics several additional performance parameters have to be met: high quality, high throughput, low costs. Here we describe the design and validation of an NGS approach for the diagnostics of inherited retinal dystrophy. We developed a 12-plex Nimblegen sequence-capture array to enrich exons, splice junctions and UTRs of 111 known retinal dystrophy genes. This array was used to enrich the determined gene package in 100 previously unsolved patients with isolated or autosomal-recessive RP. DNAs of 12 RP patients carrying 24 known disease-causing variants served as controls. All 112 samples were sequenced on a Roche454 GS-FLX Titanium. Stringent bioinformatic data-analysis and variant detection on average resulted in 4 variants per sample which were validated using conventional Sanger sequencing and subsequently tested for segregation within the family. Preliminary data on the confirmation and segregation analysis revealed the likely pathogenic mutations in at least 35 probands. These results show that our approach is operational, e.g. that the coverage obtained allows for reliable identification of the disease-causing variations. Targeted NGS combined with stringent bioinformatic data-analysis holds the promise to optimize cost-effectiveness in molecular diagnostics for genetically heterogeneous disorders like RP, and we are convinced that this approach is an important step towards the successful implementation of NGS in diagnostics.

P11.139

Mutation screening of multiple genes in Spanish patients with Autosomal Recessive Retinitis Pigmentosa using a Custom-designed Resequencing microarray

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Retinitis Pigmentosa (RP) is a heterogeneous group of inherited retinal dystrophies characterised ultimately by the loss of photoreceptor cells. RP is the leading cause of visual loss in individuals younger than 60 years, with a prevalence of about 1 in 4000. The molecular genetic diagnosis of autosomal recessive RP (arRP) is challenging due to the large genetic and clinical heterogeneity. Traditional methods for sequencing arRP genes are often laborious and not easily available and a screening technique that enables the rapid detection of the genetic cause would be very helpful in the clinical practice. The goal of this study was to develop and apply microarray-based resequencing technology capable of detecting both known and novel mutations on a single high-throughput platform. Hence, the coding regions and exon/intron boundaries of 16 arRP genes were resequenced using Affymetrix microarrays in 103 Spanish patients with clinical diagnosis of arRP. All the detected variations were confirmed by direct sequencing and potential pathogenicity was assessed by functional predictions and frequency in controls. For validation purposes 7 positive controls for variants consisting of previously identified changes were hybridized on the array. As a result of the resequencing screening, we detected 36 variants, of which 17 are very likely pathogenic. They consist of 1 nonsense mutation, 3 splicing mutations, 1 5' UTR variant and 12 missense mutations. The 19 remaining identified variants included 3 non synonymous, 4 synonymous and 12 intronic polymorphic changes. The accurate detection of the positive controls allowed the validation of the technique.

P11.140

Rheumatoid arthritis (RA) associated-gene, *CD244* expressed in RA synovial tissue

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RA is well-known as an autoimmune disease and is a chronic inflammatory disorder characterized by the destruction of multiple joints. Many genome wide association studies were performed and multiple RA-susceptibility loci and autoimmune-susceptibility loci have been identified. These studies suggested that multiple genes and its functions were related with disease causing and development. These studies also indicated an important factor regarding genetic factors of RA and autoimmune diseases; some of the RA-susceptible polymorphisms also increase the risks of other autoimmune diseases. One of the mechanisms of the inflammation in autoimmune diseases associated with signal transduction via signaling lymphocytic activation molecule (SLAM). It was reported that SLAM family gene, e.g., Ly108 is also associated with systemic lupus erythematosus (SLE). We identified CD244 gene as susceptibility gene to RA. The association peak in the block was observed at two functional SNPs (rs3766379 and rs6682654) in CD244 in two independent RA cohorts from Japan ($P= 3.23 \times 10^{-8}$ and $P=7.45 \times 10^{-8}$) and a SLE cohort. These RA-susceptible variants of rs3766379 and rs6682654 upregulate expression of CD244, their causative role to increase the risk of autoimmunity has to be further investigated for its validity and its precise molecular mechanism. Furthermore, we indicated that CD244 also expresses in synovial tissues of CIA mice and RA patients. We supposed that up-regulation of CD244 affect on RA and other autoimmune diseases, including SLE. Thus, CD244 is a novel genetic risk factor for RA and may have a role for autoimmunity in RA.

P11.141

Functional characterization of OA risk polymorphism rs225014 at DIO2 in human OA cartilage

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Previously, the deiodinase iodothyronine type-2 gene (*DIO2*) was identified as an osteoarthritis (OA) risk gene, and SNP rs225014 located within the DIO2 gene was found to be associated with OA. To date, the underlying mechanism of this association is unknown and functional characterization of alleles discriminated by this SNP using an allelic imbalance assay may give more insight in putative genetic regulatory elements underlying the association.

Cartilage RNA obtained from heterozygous individuals (N=13) was subjected to an allele discriminating Taqman assay and the relative peak heights of allele C and T fluorescence signals were assessed at both genomic DNA (1:1 reference) and RNA level. Deviations from allelic balance in the normalized log transformed RNA samples were tested against the normalized log transformed DNA samples for significance using a Mann-Whitney non-parametric test.

We show a highly significant allelic imbalance, where the OA risk allele (C) is present at higher levels in OA cartilage RNA of 13 subjects than allele T (Table 1). None of the genomic DNA's showed signs of deviations from allelic balance when tested against all remaining genomic DNA's. To further investigate the *DIO2* gene activity in OA we aim to assess absolute expression levels of *DIO2* in both healthy and OA affected cartilage samples and investigate the protein presence of DIO2 in OA and healthy cartilage.

Table 1. Allelic imbalance measurements.

Donor	Sex	Age	Joint	cDNA[1] (%)	Genomic[2] (%)	Relative fraction C/T	P-value[3]
1	F	70	Right Shoulder	18 (90)	N/A	1.30	<0.01
2	F	N/A	Left Hip	19 (95)	5 (100)	1.26	<0.01
3	M	61	Right Hip	20 (100)	5 (100)	1.29	<0.01
4	F	59	Right Hip	16 (80)	4 (80)	1.34	<0.01
5	M	71	Right Hip	19 (95)	4 (80)	1.32	<0.01
6	F	75	Left Knee	20 (100)	5 (100)	1.33	<0.01
7	F	78	Right Hip	19 (95)	5 (100)	1.25	<0.01
8	F	75	Left Hip	20 (100)	5 (100)	1.60	<0.01
9	F	79	Right Knee	20 (100)	5 (100)	1.38	<0.01
10	M	56	Left Hip	17 (85)	5 (100)	1.36	<0.01
11	F	62	Right Hip	18 (90)	5 (100)	1.41	<0.01
12	F	79	Right Hip	17 (85)	5 (100)	1.42	<0.01
13	F	62	Shoulder	17 (85)	5 (100)	1.42	<0.01

[1] *cDNA PCR measurements passing quality control (max. 20)*
[2] *Genomic PCR measurements passing quality control (max. 5)*
[3] *Mann-Whitney non parametric test (cDNA per individual versus genomic DNA samples pooled)*

P11.142

Multiplexed and Targeted next-generation sequencing for the molecular genetic diagnostics of Osteogenesis Imperfecta

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Background: Osteogenesis Imperfecta(OI) is characterized by susceptibility to bone fractures and defects in collagen type I biosynthesis. OI is caused in 90% of cases by dominant variants in 2 genes(*COL1A1*, *COL1A2*) and in ≤10% of cases by recessive variants in 7 genes (*CRTAP*, *LEPRE1*, *PPIB*, *SERPINH1*, *FKBP10*, *PLOD2, SP7*). In some cases no mutation can be found in these genes. or in other unidentified genes. Sequencing occurs stage wise, which is time consuming, and expensive.

Methods: OI patients were selected for abnormal collagen type I production without sequencing results or because of negative sequencing results with clinical/radiological information and/or protein analysis suggestive of OI types II-IV. Samples were analysed by targeted enrichment for known and candidate genes, followed by next-generation sequencing on the Illumina GAIIx platform. DNA libraries from 20 individuals were multiplexed and sequenced on a single Illumina flowcell lane using a 75 cycles paired-end protocol. An in-house variation detection pipeline was used to score for relevant mutations.

Results: An average of 2.9 million reads per sample was obtained of which 2.1 million reads (71%) mapped to the targeted regions. This resulted in an average depth of coverage exceeding 180 fold. Analyzing 12 out of 20 samples, 2 causative *COL1A1* variants and 2 causative *COL1A2* variants were discovered. Furthermore, 12 non-synonymous amino acid changes were detected, which are predicted to be harmful and may represent causative variants.

Conclusion: Multiplexed and Targeted next-generation sequencing

allows for comprehensive genetic screening of OI patients in a fast and cost-effective manner.

P11.143
Identification of genes expressed in human inner ear tissue using next generation RNA sequencing

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Characterization of the human inner ear transcriptome is important for the study of the basic biology of hearing and diseases of the inner ear. We present RNA-Seq data of the cochlea, ampulla, saccule, utricle, and total vestibular regions of the human inner ear derived from fresh surgically resected tissue from four donor patients. Tissue was collected from non-diseased regions during an operation to remove a tumor located near the inner ear. Libraries were sequenced on the Illumina HiSeq 2000 instrument with an average of 127 million quality-filtered, 104bp paired-end reads per sample. We performed mapping with TopHat to human GRCh37 and quantitation with Cufflinks. In the three sequenced cochleas, we measured expression from 20655 genes that produce 89066 total transcripts. In vestibular regions we find 21501 expressed genes with 92377 unique transcripts. Comparisons between cochlea and vestibular regions showed 1567 genes unique to the cochlea and 2413 genes unique to vestibular tissue. In total we find 23068 genes expressed in the inner ear along with 98837 total transcripts, an average ratio of >4 transcript variants per gene. Of note, Aquaporin-1 is expressed 25-times higher, FOS is 18.5-times higher, and Otospiralin is expressed 40-times higher in cochlea versus vestibular samples. Otogelin is expressed 65-fold higher in vestibular tissue over cochlea replicating previous expression studies in the mouse. These data represent one of the first high-resolution transcriptome maps of the human inner ear and may prove useful as a resource for prioritization of candidate genes for the study of hearing loss.

P11.144
RNA-seq uncovers the influence of structural variants on transcriptome diversity

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Structural variation (SV) of DNA segments has been identified as a major source of genetic diversity, but a comprehensive understanding of the phenotypic effect of these variants is only beginning to emerge. Our group and others established extensive maps of SVs in wild mice and inbred strains, covering ~11% of their autosomal genomes. SVs shape tissue transcriptomes on a global scale and thus represent a substantial source for within-species phenotypic variation. The recently described RNA-seq method has brought transcriptome analysis to a new level, as it addresses gene expression at nucleotide resolution and alternative splicing events simultaneously. We used these advantages to unravel the effects of SVs on expression. We generated by ultra high-throughput sequencing on Illumina Genome Analyzer II >450 millions RNA-seq reads from brain and liver of three mouse inbred strains (129S2, DBA/2J and C57BL/6J) to monitor expression changes of transcripts that map within and outside SVs. We used TopHat and Cufflinks to map, assemble and estimate the abundance of the different isoforms. Results show that SVs impact gene expression and splicing diversity of genes lying within, but also that of genes which present structural variation in their 5' and/or 3' regions. SV related genes are enriched among isoforms differentially expressed between strains (p-val = 5e-08 and 8e-04 in 129S2 and DBA/2J respectively).
This study provides a unique opportunity to extensively gauge the influence of structural variants on the transcriptome complexity and regulation.

P11.145
Contrasting features of the close homologues SERPINA2 and SERPINA1

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Serine protease inhibitors (SERPINs) are a superfamily of highly conserved proteins that play a key role in controlling the activity of proteases in diverse biological processes. In humans, the largest SERPIN cluster is located at 14q32.1 region and includes the gene coding for the archetypical SERPIN, SERPINA1, and a highly homologous sequence, SERPINA2, which was originally thought to be a pseudogene. However, several lines of evidence collected in our work have identified SERPINA2 as a polymorphic gene, probably encoding a functional protein with unknown inhibitory activity. Previously, we have found that SERPINA2 could carry a 2kb deletion leading to an inactive SERPINA2 that is likely to be associated with a signature of positive selection in Africa. To further evaluate SERPINA2 functionality we performed several molecular assays in cell lines stably expressing different SERPINA2 and SERPINA1 variants. In contrast to SERPINA1 recombinants, which were found at both intra- and extra-cellular levels, SERPINA2 was detected only inside the cells. These results differ from the expected patterns of Clade-A SERPINs, which are normally secreted and retained in the cells, within the endoplasmatic reticulum (ER), in case of abnormal folding. Still, assays of proteosome and fagosome inhibition resulted in similar patterns for SERPINA2 and SERPINA1, suggesting that both recombinants expression is down-regulated by post-translation mechanisms mainly involving ER-associated degradation. Although enzymatic assays are still needed to determine the inhibitory properties of SERPINA2, our findings provide the first in vitro evidence for SERPINA2 expression and suggest an activity distinct from its closest homologue, SERPINA1.

P11.146
Effects of a dietary intervention with red wine, dealcoholized red wine and gin on the expression of *SIRT1* in human peripheral blood mononuclear cells

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Sirtuins are becoming increasingly recognized as important epigenetic drug targets in aging and several metabolic diseases. The first sirtuin identified was silent mating type information regulation-2 homolog (SIRT1), considered a longevity gene. Resveratrol, a polyphenol found in red wine, is known to activate *SIRT1*. Various studies have demonstrated that resveratrol extends the life span of diverse species through activation of *SIRT1*. On the other hand, some studies have reported that alcohol intake can also increase *SIRT1* expression. Our aim was to investigate the effects of a dietary intervention with red wine (resveratrol+alcohol), dealcoholized red wine (resveratrol) and gin (alcohol) on the expression of *SIRT1* in humans. 73 men (60+/-8 years) were enrolled in a crossover study in which three dietary interventions (28 days each) were randomized. During these interventions the same subjects must take 100 ml of gin (or 272 ml of red wine or 272 ml of dealcoholized red wine per day. We analyzed *SIRT1* expression in 66 subjects who completed the trial. Total RNA was extracted from peripheral blood mononuclear cells (PBMC). RNA levels were measured with real-time PCR and normalized using the Ct method of relative quantification. We checked 31 housekeeping genes, and *GADPH* was used. We observed that the dietary intervention with red wine, dealcoholized red wine and gin statistically increased *SIRT1* gene expression (about a 11%) compared to baseline levels (1.14+/-0.5 vs 1.03+/-0.03; P=0.008). In conclusion, our results suggest that both resveratrol and ethanol contribute to increase *SIRT1* gene expression in human PBMC.

P11.147

Architecture and patterns of population differentiation of SNPs in drug response pathways

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Response to drugs varies amongst different populations. We hypothesized that these differences in drug response are influenced by Single-Nucleotide-Polymorphisms (SNPs) that are highly differentiated amongst the different populations. We thus examined the SNP architecture and patterns of population-differentiation (F_{ST}) of SNPs in genes of drug-response-pathways (DRP) in the human genome using a gene-based 'functional region-directed approach. A total of 500,000 SNPs were found to localize to 715 genes of 66 DRPs. The 5'/3'UTRs of genes in most DRPs exhibit significantly higher SNP density than the average genome SNP density while the coding region showed the reverse trend suggesting that the UTRs of DRP genes are more polymorphic while the coding regions are more constrained and highlighting that differences in drug response may be more likely due to differences in the expression of these genes and not to differences in the protein structure/function. Significantly, non-synonymous coding-SNPs of 2 DRPs of drugs involved in vasoconstriction and ischemic heart diseases were found to be significantly population differentiated suggesting that population differences in response to these drugs may be due to population differences in SNPs of these pathways. Notably, SNPs in most regions, except promoter and 3'UTR, of the genes involved in drugs affecting the Sympathetic Nerve Pathway (SNPy) exhibit significant population differentiation suggesting that there may be population differences in response to drugs targeting this SNPy pathway. Hence this study highlights potential population differences in the response to drugs that target specific pathways with high F_{ST}.

P11.148

A SNP array evaluation tool to identify autosomal recessive conditions in the offspring of consanguineous unions.

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Introduction.
Mendelian disorders for which the genetic basis has been identified is increasing rapidly. These disorders pose a challenge to the clinician to diagnose. In a consanguineous union, the offspring has various runs of homozygosity (ROHs), identifiable by single nucleotide polymorphism (SNP) array technology. We hypothesized that an evaluation tool that identifies all genes associated with OMIM-annotated AR conditions in ROHs of a patient will allow the clinician to compare the patient's clinical features with the phenotypes of these autosomal recessive (AR) conditions, making a SNP-array the preferred diagnostic approach.
Approach/Results.
We developed a Perl-CGI online evaluation tool that allows the clinician to identify genes associated with OMIM-annotated AR conditions in ROHs obtained from SNP-array. It incorporates the OMIM database and other genetic databases, providing the user with a summary of genes and pertinent genetic information mapping to the query ROHs. The scale of gene search on the query ROHs can be adjusted. We have tested this tool using cases from our clinical practice. In this manner we diagnosed known AR conditions that had remained unrecognized until employment of this tool.
Conclusions.
SNP-array can be employed to determine the ROHs in the setting of consanguinity. These ROHs can be evaluated for OMIM-annotated AR conditions. We have developed a genomic SNP-array evaluation tool allowing the clinician to evaluate for these AR conditions. We have demonstrated its usefulness in clinical practice, and believe that the SNP-array, together with this Perl-CGI evaluation tool, will change the diagnostic approach in the setting of consanguinity.

P11.149

An integrative bioinformatics pipeline for structural variation analysis of whole-genome sequencing data

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Recent introduction of high-throughput sequencing technologies has increased research activities in efficient computational tools for the identification of structural variants (SV) and their association with human diseases. We have designed a pipeline (PeSV-Fisher) for the analysis of paired-end and mate-paired sequencing data, using a combination of geometrical paired-end mapping strategies (PEM) and depth-of-coverage analysis(DOC), and focused on the identification of somatic variation. It is capable of analyzing several samples simultaneously and provide a list of variants and their carriers.
PEM is used for the identification of four types of SV: insertions, deletions, inversions and translocations. Read-pairs with a phred-based quality score over 35 are classified into four categories: (1) correct order and orientation (insertions, deletions and normal); (2) wrong orientation (inversions); (3) mapping to different chromosomes (translocations); and (4) unpaired or unmapped. The empirical distribution of the insert size is calculated from the first category, and used to define cut-off sizes to discriminate read-pairs supporting insertions or deletions. Then, a clustering procedure to group read-pairs pointing to the same SV is performed. Breakpoints of presumed SV are predicted from each cluster containing at least two read-pairs.
Further support for identified variants is obtained by DOC analysis. The genome is fragmented into non-overlapping 100 nt intervals. The number of sequences mapping to each fragment is counted and normalized based on GC-content. This values are fed into a segmentation algorithm. In addition, sequences from the 4th category mapping around the breakpoints of the predicted SV are checked for split reads using GEM.

P11.150

The new method for quantitative tracking of T cell clones.

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The individual repertoire of T Cell Receptors (TCRs) is a mirror of functioning of an immune system that keeps detailed information concerning infectious and autoimmune conditions. Here we have developed a new approach that enables unbiased quantitative analysis of the human TCR V beta repertoire by mass sequencing. The method based on direct amplification of TCR cDNA with universal primers with subsequent next generation sequencing. Two next generation sequencing platforms were compared showing good correspondence of obtained results. We employed the new method to perform detailed long-term tracking of the fate of T cell clones after high-dose chemotherapy and autologous hematopoietic stem cell transplantation (HSCT). We show that multiple clones survived the transplantation procedure, some of them being essentially suppressed, but some of them expanding and fighting infections early after HSCT. We believe that wide application of the proposed approach will lead to better understanding of infectious and autoimmune conditions, and development of the individual sequence-based diagnostics of immune status.

P11.151

Epimutations in teratocarcinoma development

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Testicular germ cell tumors (TGCTs) are the most frequent cancers in young men. Present study takes advantage of the experimental mouse teratocarcinoma (TCa) model, a powerful tool for detecting /monitoring alterations, including epigenetic disruptions, during a timeframe of cancer development in vivo.
TCas were obtained by transplanting 7,5-days-old C3H embryos under the kidney capsule of syngeneic adults. Animals were killed after 2, 4, 6 or 8 weeks. Bisulfite modification of gDNA was performed and DNA methylation status was analyzed by pyrosequencing assay.

TCas exhibited two time points of intensive growth; in the 4th and 8th week. Significant changes in Oct3/4 DNA methylation were found during 8 weeks TCa development. From the 4th week, TCas showed significant Oct3/4 hypermethylation when compared to testicular tissue. Only 8 weeks old TCas showed strong negative correlation between Oct3/4 DNA methylation and TCa growth. Significant changes in DNA methylation of Nanog promoter were found during 8 weeks TCa development. All TCas showed significant Nanog hypomethylation when compared to testicular tissue. We noticed negative correlation between TCa weight and Nanog DNA methylation in 4 weeks old TCas. Scgb3a1 and Prss21 showed constant hypermethylation in TCas when compared to normal testicular tissue.We suggest that Nanog DNA hypomethylation represents an early cancerogenic force pushing cells into TGCT/teratocarcinoma development while Oct3/4 DNA hypermethylation seems to compensate cancer initiation and inhibit cancer progression. If Oct3/4 is not methylated, intense cancer progression can take place. Genes involved in testicular differentiation seems to remain suppressed during TCa cancerogenesis.

P11.152
Genetic variation in monozygotic twins

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Twins are defined as two births from a single pregnancy. Fraternal, or dizygotic twins, are derived from separate fertilization events and share on average 50% of their DNA, as do any other siblings. However, approximately one third of twins are derived from a single egg that splits post-zygotically. These are referred to as identical or monozygotic twins, and are considered to be genetically the same. Based on this assumption, twin studies have been considered a powerful tool for determining the contribution of genetic versus environmental factors to specific phenotypes.

In recent years there have been reports suggesting that genetic differences within twin pairs due to somatic mosaicism may have been underestimated. To explore this further we have performed copy number variation (CNV) analysis on DNA from four different sources - placenta, buccal swabs, umbilical vein vascular endothelial cells and cord blood mononuclear cells - from four different monozygotic twin pairs (32 samples in total) using the Illumina HumanOmni1-Quad beadchip.

To date we have identified a number of potential mosaic CNVs, either between tissues within an individual or between individuals of a twin pair. We are currently confirming these findings using different molecular techniques such as high resolution melting curve analysis, and are looking at single nucleotide variant differences using next-generation sequencing.

P11.153
SNP array study of uniparental disomy in Estonian mental retardation patients and general population

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Detecting loss of heterozygosity or uniparental disomy (UPD) with whole-genome SNP arrays might be as informative as copy-number data in unexplained cases of genomic disorders. Although these copy-neutral rearrangements have not been extensively studied at whole-genome level so far, it has been shown that SNP microarrays are reliable in detecting known UPD regions.

We report a cohort of Estonian patients with idiopathic mental retardation (MR) and their unaffected family members screened by Illumina Infinium HD whole-genome genotyping arrays. To detect possible inconsistencies in Mendelian inheritance as indicative in UPD, we compared genotype data for 36 parent-offspring trios. As a reference we used 48 parent-offspring trios provided by the Estonian Genome Centre at the University of Tartu to investigate possible UPD regions in general population by applying the same genotyping platform and UPD detection approach. A patient diagnosed with Prader-Will syndrome, confirmed by microsatellite analysis to have maternal UPD 15, was used as a positive control.

To evaluate the UPD detection approach, we confirmed heterodisomy along the long arm of chromosome 15 in case of the control patient, but also revealed a maternally inherited isodisomic segment encomapssing the region. Using genome-wide high-density SNP markers gives an advantage over locus-specific analyses for detecting UPD events and describing the mechanisms of formation. So far our results in general population and MR trios suggest that UPD is not extensively present in the human genome. A similar outcome has previously been reported in other cohorts of patients with idiopathic MR by SNP arrays.

P11.154
Haploinsufficiency of the CLIP2 gene is not the cause of visuospatial impairment in Williams-Beuren syndrome

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Williams-Beuren syndrome (WBS) is a contiguous gene deletion syndrome, associated with a recognizable facial appearance and an unusual cognitive profile. In total 26-28 genes are located in the commonly deleted region on chromosome 7q11.23 that is 1.5 Mb in size. The study of atypical deletions and mouse models with deletions of individual genes has enabled limited unravelling of the relationship of the individual genes to each of the clinical features. Three genes are believed responsible for the Williams-Beuren syndrome cognitive profile, including LIMK1, CLIP2 and GTF2I(RD1/2) , yet no specific deletions or mutations in any of these 3 individual genes have been described. A major role for the CLIP2 gene in the cognitive characteristics of WBS has been suggested in reports of patients with partial deletions of the WBS region not including CLIP2 , that displayed only mild or no visual-spatial impairment.

We describe here two patients with deletions taking away exon 3-15 of the CLIP2 gene in two sibs. Specific deletions of the CLIP2 gene have not been reported before. Cognitive testing of both sibs revealed an intelligence in the normal range and showed no evidence for deficits in visual-spatial construction. These data make it highly unlikely that haploinsufficiency of CLIP2 on its own causes the cognitive disabilities associated with this syndrome. The cognitive profile in the Williams Beuren syndrome can therefore not be explained by dissecting the contribution of individual genes but has to be considered the consequence of the interaction between several genes within the deleted region.

P11.155
New clinical delineation of the 14q11.2 duplication syndrome based on a new case and the contribution of maternal UPD14 in the previous patient

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In 2007 we reported a de novo duplication of 14q11.2
(5.38 Mb) associated to short stature, mild mental retardation, hypotonia and minor dysmorphic features. Later the complementary microdeletion syndrome was characterized and narrow down to ~35 kb, with SUPT16H and CHD8 as the best candidate genes. Here we present the new clinical delineation of the 14q11.2 duplication syndrome based on a new case, as well as on the recent finding that the previous patient also showed maternal uniparental disomy 14 (UPD14). In consequence, many of the clinical features previously associated to 14q11.2 duplication, were indeed due to maternal UPD14. These features include prenatal and postnatal growth retardation, hypotonia, orthopedic problems, motor delay, early onset of puberty and minor dysmorphic features of the face.On the other hand, array-CGH analysis of a new patient identified a *de novo* duplication of 4.62 Mb in 14q11.2 (18679179-23294903; hg18). She presented expressive language delay, no motor delay, behavioral problems and minor dysmorphic features: hypothelorism, bushy eyebrows, flat nasal root, highly arched palate, bilateral genu recurvatum and dorso-lumbar kyphosis. Other clinical features, such as growth parameters or IMR, were normal at examination. Based on the common clinical signs between both patients, we can conclude that 14q11.2 duplication only associates a

mild developmental delay and minor dysmorphic features, but no or very mild cognitive impairment.

P11.156
The Efficiency of Double-Stranded DNA Formation in cDNA synthesis can be Crucial for Precision of Microarray Expression Analysis

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Introduction. The quality and reproducibility of microarray expression experiments within and between laboratories are compromised by the complexity of the samples and procedures. We used Two-Dimensional Strandness-Dependent Electrophoresis (2D-SDE) to investigate the quality and reproducibility of the cDNA synthesis. 2D-SDE can measure double-stranded cDNA the essential template for T7 RNA polymerase.

Material and Methods. SuperScript III reverse-transcriptase was used to synthesize cDNA from Universal Human Reference RNA (Stratagene) using T7(dt) primer. The samples were synthesized both according to kit protocol and according to our improved methodology. The amount of dsDNA was mesured with 2D-SDE. The cDNA was transcribed with T7 RNA Polymerase and the labeled aRNA hybridized to Agilent microarray containing probes from 230 genes. Each gene was represented by 10 probes and each probe was replicated six times to estimate the variability of the experiments. Results were analyzed using the R software.

Results. The amount of double-stranded cDNA after synthesizing the same RNA samples six times was very variable ranging between 0 and 73%. Microarray experiments showed that the higher the dsDNA percentage the fewer probes had significant variability between samples. Approximately 15% of probes showed variability (p = 0,05) between samples when the dsDNA percentage was between 12% and 35%. In contrast, only 3% of probes showed variability between samples when the dsDNA percentage was 69% and 73%.

Conclusions. Our results indicate that an important component in imprecision in T7 RNA polymerase-based microarray expression analysis can be explained by the amount of double-stranded cDNA synthesized.

P11.157
Array-DASH: Dynamic microarrays for broad utility in DNA fingerprinting, genotyping, and re-sequencing in diagnostics

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Despite the emergence of ultra-high-throughput DNA analysis platforms, it is probable that the need for low cost and simple/automated methods that robustly assay specific fragments of DNA will remain. Examples include DNA diagnostics of infectious disease, Mendelian disease, certain cancer situations, environmental monitoring, and forensics. In such cases, microarrays offer a convenient and flexible way of analysing DNA, but suffer from imperfect fidelity if hybridisations are conducted at a single stringency. Arrays can, however, fully resolve all short DNA sequence variations in all sequence contexts if hybridisation and/or washing entails a dynamically changing temperature range with real time signal monitoring.

This 'Dynamic Allele Specific Hybridisation' (DASH) principal requires interpretation of surface-phase melt-curves, enabling DNA fragments to be re-sequenced with high precision compared to standard arrays, in both a quantitative and sensitive manner (alleles detectable at a few percent of the input DNA).

Current work fuses the original plate-based DASH approach, with the semi-automated HybLive microarray platform (Genewave) and on-slide oligo synthesis using the FlexArrayer (FlexGen). Single base variants, small indels, and microsatellites are resolved with melting temperature differences of up to ~10°C. 'Array-DASH' is now being assessed for real world diagnostics applications involving re-sequencing, multiplexed mutation scoring, regional scanning for mutations, and combinations of the above - providing a single, generic platform for convenient analysis of clinical and other DNA samples in a multitude of scenarios.

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P11.158
Transcriptome analysis in haematopoiesis in development and disease

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Platelets are the second most abundant cell in the blood and are derived from haematopoietic stem cells (HSCs) through a process named megakaryopoiesis. The process of platelet formation by polyploid megakaryocytes (MKs) in the bone marrow is tightly regulated and each MK produces about 3000-6000 platelets. The anucleated platelet plays an important role in maintaining haemostasis by surveying the vessel wall for damage and if identified initiate repair. Rare inherited Mendelian disorders of platelets may be associated with impaired platelet function, abnormal platelet numbers and/or volume and some of these disorders are associated with a propensity of bleeding.

Studying the transcriptome of platelets is challenging given that platelets survive only nine days on average and their mRNA degrades rapidly. We have therefore used high-throughput technologies (microarrays and next generation sequencing platforms) to gain a comprehensive insight in the repertoire of actively expressed RNA transcripts in megakaryocytes, the precursors of platelets.

In this study we have applied high-throughput sequencing of RNA from human MKs that have been obtained by *in vitro* culture of purified CD34+ HSCs in the presence of thrombopoietin and interleukin 1β. We integrate these data with ChIP-seq data of two key transcription factors in megakaryopoiesis and platelet formation (MEIS1 and NFE2) in order to study genome-wide regulatory targets and their role in driving lineage-specific transcription in megakaryocytes.

P11.159
Parallel Detection of Copy Number Variation and Loss of Heterozygosity with CGH Microarrays

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High resolution array CGH has been widely used to investigate DNA copy number variation (CNV) associated with complex disorders. Disease association studies have become increasingly focused on CNVs, and several recent reports show links between CNVs and schizophrenia, autism, and cancer, among others. However, many copy neutral events undetected by conventional array CGH, including copy neutral loss of heterozygosity (LOH) and uniparental disomy (UPD), also contribute to disease phenotypes. Regions of the genome absent of heterozygosity are usually determined through the detection of single nucleotide polymorphisms (SNPs) for which each parent has contributed a different allele.

Until recently, cytogenetic research needing both high resolution, high quality copy number analysis and LOH detection required two separate array platforms. At Roche NimbleGen we have developed a combination array CGH/LOH detection platform by adding probes that respond to SNPs to existing array CGH designs. Two probes detect each SNP location, including a perfect match probe (A allele) and a mismatch probe (B allele) similar to our comparative genomic sequencing platform. Using our patented probe design strategy, the detection of over 600,000 common SNPs (MAF >0.4) was tested to ensure the best responding SNP probes were added to the array CGH designs. We will show data from a new 6X630K platform that allows high resolution and high sensitivity detection of both CNVs and LOH following the standard CGH workflow. Samples with known regions of UPD were used to assess the performance of this new design with a resolution for detecting LOH at approximately 1MB.

J11.01
Investigating the effect of single nucleotide polymorphisms at -7 & -138 positions on MGP gene promoter activity

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The matrix Gla protein (MGP) is an important inhibitor of vessel and cartilage calcification that is strongly expressed in human calcified, atherosclerotic plaques. Two single nucleotide polymorphisms (SNPs) at -7 & -138 positions on MGP gene have been suggested to play a role in susceptibility towards Coronary Artery Disease (CAD). Such a role is possibly due to the effect of these SNPs on the level of gene expression. The aim of this study was to investigate the possible effect of -7 and -138 SNPs on MGP gene promoter activity *in vitro*.

To achieve this aim, four appropriate vectors that express GFP under the control of different haplotypes of MGP promoter have been constructed. To assess the effects of haplotypes on promoter activity, vectors were transfected into the Hek293 cells and relative expression of GFP was quantified. Analysis of data using Student's T-test, revealed that G-7A SNP has a significant effect on promoter activity in cultured cells. Presence of nucleotide A in -7 position resulted in 12% increase in promoter activity (p<0.002). T-138C SNP did not show a significant change in promoter activity (p>0.7). The maximum difference in promoter activity is seen between vector containing A-C and G-T haplotypes (p<0.0005). Less significant differences in promoter activity were shown between A-T and G-T haplotypes as well as A-T and G-C haplotypes (p<0.05).

J11.02
Genetic analysis of HTR2A and HTTLPR polymorphisms in Russian population using biochips.

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Previously it was show that both serotonin 2A receptor (5-HTR2A) and serotonin transporter linked promoter region (HTTLPR) were important for normal functioning of the brain. Molecular genetic studies revealed that HTTLPR and 5-HTR2A polymorphisms were associated with schizophrenia and possibly influenced clinical outcome or response to treatment.

The objective of this study was to develop a biochip to determine serotonergic system genes polymorphisms for further clinical application. A biochip allows to analyze a spectrum of allelic variants in two different genes such as HTR2A (rs6311, rs6313, rs6314 and rs7997012) and HTTLPR (rs28914832). To estimate the accuracy of the method we examined 120 DNA samples of healthy individuals in Russian population. The results of hybridization on biochip showed 100% coincidence with direct sequencing. The genotype distributions were in Hardy-Weinberg equilibrium. The frequencies of serotonergic system gene alleles in healthy individuals are compared with allele frequencies in patients with schizophrenia to find out susceptibility allelic variants of the disease in Russians.

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J11.03
Exceptional human core promoter nucleotide compositions

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The proximal promoter sequences contain basic motifs for the expression of the downstream genes. We present genome-scale computational analyses of the 120-bp immediate upstream sequences to the +1 transcription start sites (TSSs) of 10,117 human protein-coding genes, and unravel exceptional genes in respect with the core promoter nucleotide composition. Our data reveal that while in 99% of the genes the absolute purine/pyrimidine ratio ranges between 0.2 - 2.5, certain genes show exceptional skew in this balance (e.g. ratios of 82.3 in VWA3A, 61.5 in Sox5, and 24.0 in BRWD3), and consist of islands of purines or pyrimidines. Furthermore, while over 95% of the genes lack more than one short tandem repeat (STR) in their core promoters, certain gene promoters are exceptionally rich in multiple STRs (e.g. eight consecutive STRs in UBE2QL1, and six STRs in GRIA2). We found sequence bias for the majority of those promoters across species, supporting functional roles for them in gene expression. Genes downstream to those promoters were also found to be of ontologic importance (i.e. we were able to track the majority of those genes to the lower species such as S. Cerevisiae and C. elegans). The exceptional promoters presented in this study lack the conventional motifs for the TATA, and TATA-less promoters, hence offering novel mechanisms for gene expression. They may also provide potential mechanisms for inter-individual variations in gene expression, and complex traits/disorders.

J11.04
Screening of three mtDNA mutations using PCR-RFLP technique a province of Iran

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Non-syndromic hearing loss may be induced by mutations in both nuclear and mitochondrial genes. Mutations in mtDNA are present in less than 1% of the children with pre-lingual deafness but are more prevalent later. Most of the molecular defects responsible for mitochondrial disorder, associated with hearing loss may be induced by mutations in the 12SrRNA and tRNA genes. This aim of this study was to investigate the frequency of three common mtDNA mutations including A1555G, A3243G and A7445G in a cohort of autosomal recessive non-syndromic hearing loss (ARNSHL) subjects in Sistan va Baluchestan province.

Material and Methods: In this descriptive- experimental based study, a total of 110 **Methods:** ARNSHL subjects from Sistan va Baluchestan province were investigated for three common mtDNA mutations using PCR-RFLP procedure. The possible mutations were confirmed by direct sequencing.

Results: None of the A1555G and A7445G mutations were detected in this study. However, we found one sample to carry A3243G mutation (0.9%). Moreover abolishing a MTTL1 restriction site close to A3243G mutation revealed a G3316A allelic variant in 0.9% of patients studied.

Discussion: This study showed that mtDNA mutations are responsible for less than 1% of pre-lingual ARNSHL associated subjects. The present study will improve the genetic counseling of hearing impaired patients in Sistan va Baluchestan province, Iran.

J11.05
Study of genetically determined production of interleukin-1b

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Revealed increasing levels of interleukin-1 beta (IL-1b) have "conditional" healthy people in the republic of Bashkortostan.

Investigated 180 "conditionally" healthy individuals. The level of production IL1b sample was divided into groups: Group 1 (n = 96) - product IL1b to 10 pg / ml, group 2 (n = 56) - from 10 to 50 pg / ml, group 3 (n = 28) - above 50 pg / ml.

To identify genetic characteristics of the regulation of production IL-1B, have studied the distribution of genotypes and alleles of the family, interleukin-1 in the three groups studied: IL1b (C3953T), an antagonist of IL-1 receptor - IL1Ra (VNTR-polymorphism), IL-1 receptor - IL1RI (T976C).

Results. In group 1, revealed a high frequency of combinations of

genotypes E1E1 / / I / I / / CC (p = 0,05, χ2 = 3,7), it provides a low production and reception of IL-1b, high competition for the cytokine receptors with anatagonistom. Balance between the production, expression and inhibition of synthesis of IL-1 plays a key role in the course of the inflammatory process.
In group 2, and 3 revealed a high frequency of combinations of genotypes E1E2 / / I / II / / TC (p <0.05), whereas in group 1 is the combination does not occur. This interaction between genotype causes high production and reception of IL-1b, and also promotes the launch of a cascade of reactions involving other cytokines.

J11.06
Establishment of stable CHO cell line, expressing recombinant HO-1 and study of its cytoprotective property'

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Exposure of mammalian cells to oxidative stress conditions induces Heme Oxygenase-1(HO-1) as well as 32-KD a heat shock protein. Heme oxygenases are rate- limiting enzymes in Heme degradation. Three distinct isoforms of Heme oxygenase have been described: HO-1, HO-2 and HO-3.Application of pharmacological agents and genetic probes to manipulate HO has brought about to new insight into the complex relationship of the Heme-HO system with the biological and pathological system under investigation. The strategies used to target HO-1 offer promising therapeutic opportunities for the effective treatment of many diseases, including diabetes, obesity, hypertension, heart disease, cancer, autoimmune disease and even allograft rejection.
In our study ho-1 gene was cloned to pcDNA3.1 plasmid by using genetic engineering method. The recombinant vector was transfected to CHO(Chinese Hamster Ovarian) cell line in order to establish stable cells expressing ho-1 gene and the expression of HO-1 confirmed by RT-PCR .Then CHO-HO-1 cell lines were treated by variety of H_2o_2 concentrations in different times compared with CHO-pcDNA3.1 and the cell viability was measured by MTT assay.
The results indicated that the expression of ho-1 lead to cytoprotective effect since the CHO-ho-1 cells showed higher viability rate compared with the control cells. On the other hand the Flow-Cytometric test revealed that the ho-1 has anti-apoptotic effect as the CHO-ho-1 cells had delay in promoting apoptosis after H_2o_2 treatment in comparison with CHO-pcDNA3.1 cells.

J11.07
SCN1A gene polymorphism in Iranian patients with migraine

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Introduction: Migraine is a prevalent and debilitating disease affecting a large proportion of the population. Mutations in three genes (CACNA1A, ATP1A2 and SCN1A) have been detected in familial and, more rarely, in sporadic cases with migraine. Here we report the molecular analysis for 2 SNPs in SCN1A gene in affected Iranian patients with migraine.
Material and Methods: Patients with clinical diagnosis of migraine according to the international headache society criteria entered in the study. SCN1A gene polymorphisms (rs7601520 and rs2298771) were investigated using direct sequencing method.
Results: Genotypes for rs7601520 were AG, AA and GG in 23 (56.1%), 14 (34.15%) and 4 (9.76%) respectively. Allele frequency were A and G in 51 (62.2%) and 31 (37.8%) respectively. Genotypes for rs2298771 were AG, AA and GG in 26 (57.8%), 15 (33.3 %) and 4 (8.9%) respectively. Allele frequency were A and G in 56 (62.2%) and 34 (37.8%) respectively.
Conclusion: Genotype and allele frequency were in the range of other studies. Further analysis f other common SNPs in this gene and in comparison with healthy controls could be helpful to study association with the risk of migraine.

J11.08
In silico and ex vivo analysis of two chimeric polytope constructs for applications in dendritic cell-based cancer immuno-genetherapy

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In silico techniques are suited for discovery of new and development of existing vaccines. Cancer immunization using the epitopes derived from the processing of proteins that are preferentially expressed on tumor cells appears to be a reasonable approach to induce antitumor immunity aimed at achieving therapeutic advantage. It would be beneficial to use T cell epitopes from more than one tumor Ag to increase disease coverage and prevent the emergence of tumor-escape variants. In current study, the related sequences of HLA-restricted immunogenic epitopes for some cancer/testis antigens which are highly overexpressed in cancers were obtained from Genbank. Two chimeric sequences were constructed by fusing the selected epitopes using hydrophobic amino acid linkers. The *in silico* gene analysis and multi parameter gene optimization of the synthetic chimer genes were performed using Stand-alone softwares. The mRNA and recombinant protein secondary-structures were predicted and 3D structural stability of the synthetic proteins were analyzed. The VaxiJen server was used to predict the immunogenecity of the whole antigens. Finally the desired properties were verified by Gen-Script (NJ, USA) and the multimeric genes were synthesized. Monocytes-derived dendritic cells (DCs) of a healthy donor were electroporated with synthesized chimeric genes. T cells were primed with transfected DCs and lytic effects of the generated CTL were measured with Cytotoxicity assay. Based on *in silico* and primary *ex vivo* results, this is promising that the structural models for chimeric genes from antigenic epitopes of CTAs which are presented may define accessibility, solubility and immunogenicity for applications in cancer immuno-genetherapy.

P12 Molecular basis of Mendelian disorders

P12.001
Targeted re-sequencing of the 22q11 region in atypical DiGeorge patients.

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The 22q11 deletion syndrome, also called the DiGeorge syndrome (OMIM 188400) is the most common chromosomal deletion syndrome in humans with an incidence of 1 in 2-4000 live births. Although the clinical presentation of 22q11 syndrome is variable, the major clinical characteristics of the syndrome are the intellectual disability, congenital heart anomalies, velopharyngeal abnormalities and characteristic facial appearance. Also these patients are at significant risk for psychiatric disorders. In addition, to the variability amongst patients with the “typical” phenotypic features, occasionally (about 1/100) 22q11 deletion carriers present atypical malformations. Despite intensive studies it remains unclear what causes this phenotypic variability of patients with the same deletion. We hypothesize that the distinct rare features are caused by unmasking a recessive allele which occurs at low frequency in the general population. In our study we focused on 24 patients with 22q11 deletion and one of the phenotypic features outside the traditional 22q11 spectrum, like anorectal malformation, arthrogryposis, polymicrogyria, eye anomalies, inner ear malformations and laryngeal web. For identification of the functional role of genes within the common deletion we captured coding parts of the remaining 22q11 region using custom designed Nimblegen capture arrays, and re-sequenced the enriched samples with 454 GS FLX Titanium chemistry. Analysis was performed based on comparison of variants present in patients with the same phenotypic feature against variants found in patients without that characteristic. The candidate genes responsible for particular features will be presented.

P12.002
A 14kb homologous deletion in intron2 of DMRT1 in two sisters with 46,XY karyotype

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We describe two sisters with 46,XY karyotype in a consanguineous family manifested primary amenorrhea, no onset of puberty, no secondary sexual characteristics, small immature uterus and gonadal dysgenesis. Mutations in known candidate genes including SRY, SOX9, DAX1, DMRT1, SF1, WT1, CBX2, DHH, SOX8 had been excluded. Genome wide copy number changes were analyzed by running on SNP 6.0 arrays and a about 14kb homologous deletion in intron 2 of DMRT1 was identified, PCR spanned the junction and gap revealed healthy parents were heterozygous carriers. Using comparative genomics in vertebrate organisms, generated by PhastCons on sequence alignments created by MULTIZ, we found several evolutionarily conserved segments in this 14kb region. Further study on the potential effect of this noncoding sequence on DMRT1 expression is undergoing.

P12.003
Aarskog-Scott syndrome : Report of the first duplication in the *FGD1* gene.

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Aarskog-Scott syndrome (AAS), a rare X-linked condition characterized by skeletal and genital anomalies, is caused by mutations in the faciogenital dysplasia 1 (*FGD1*) gene. To date 27 distinct point mutations and 2 deletions have been described. Here, we report on a male patient presenting with highly suggestive clinical features of AAS (postnatal growth retardation, widow's peak, hypertelorism, short nose, short broad hands, camptodactyly, cryptorchidism and shawl scrotum). No mutation was found by sequencing of the entire coding sequence of the *FGD1* gene. Quantitative PCR and cDNA sequencing analyses revealed a large out-of-frame tandem duplication of exons 2-12 which probably occurred by unequal recombination between two *Alu* sequences. This maternally inherited duplication implicates the RhoGEF (DH or Dbl homology) and the first plekstrin homology (pH) domains and is predicted to be a loss-of-function mutation. This is the first duplication described in the *FGD1* gene and systematic screening of large rearrangements should be included in the diagnostic strategy of AAS.

P12.004
ADRB2 gene polymorphisms and the ocular hypotensive response to topical betaxolol in Mexican patients with open angle glaucoma.

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Glaucoma is defined as a disease characterized by loss of retinal ganglion cells, excavation of the optic nerve head, visual field defects and eventually blindness, being the second leading cause of blindness worldwide. The main risk factors include elevated intraocular pressure, age, ethnicity, family history and myopia. camenteiccionario - Ver diccionario detallaB-adrenergic receptors. ADRB2 (B-2 adrenergic receptor) is expressed in the ciliary body, trabecular meshwork and wall of the vasculature of the optic nerve. Betaxolol is a beta adrenergic blocker that acts selectively on beta 1 receptors, at eye level, it is assumed that betaxolol inhibits the adrenergic tone and therefore the formation of aqueous humor in the ciliary body. The present study aimed to determine the association between the Arg16Gly and Gln27Glu polymorphisms of the *ADRB2* gene and the ocular hypotensive response to topical betaxolol in Mexican patients with open angle glaucoma. Thirty-three patients were genotyped at the Arg16Gly and Gln27Glu polymorphisms. Demographic data, disease severity, ocular pressure tests and betaxolol medication usages were recorded for each patient. The frequencies of the Arg16 and Gln27 alleles were found to be 48.8% and 72.07.%, respectively. Two haplotypes were estimated, i.e. Arg-Gln, Gly-Gln with frequencies of 5 (15.2%), 10 (30.3%). We found no statistical differences in the outcome of betaxolol treatment between the different haplotypes. We consider that other genetic factors, apart from ADRB2 polymorphisms, are involved in the outcome to the hypotensive response to topical betaxolol .

P12.005
Patients with Alagille syndrome and large deletions in JAG1 gene.

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Alagille syndrome (AGS) is a complex autosomal-dominant multisystem disorder with a highly variable expressivity. The main phenotypic features include chronic cholestasis caused by paucity of intrahepatic bile ducts, cardiac disease (mainly peripheral pulmonary stenosis), posterior embryotoxon in the anterior chamber of the eyes, vertebral defects and a typical face characterized by a triangular shape with prominent forehead, broad nasal bridge and a pointed chin. Current screening methods allow for identification of mutations in 70-90% of individuals with AGS. Most of cases are caused by intragenic point mutations in the JAG1 (Jagged1) gene localized on chromosome 20p12. A minority of AGS patients (3-6%) have microdeletions of chromosome 20p12.

Here we present 6 Polish cases of Alagille syndrome with deletions comprising a number of JAG1 exons or the entire JAG1 gene. Analysis was performed by multiplex ligation dependent probe amplification using SALSA MLPA KIT JAG1 (MRC-Holland). In two cases deletions were inherited from one of the parents who presented with minimal symptoms. All 6 cases presented with classical symptoms: cholestasis due to paucity of bile ducts, cardiac defects, eye and vertebral anomalies and typical facies. One patient was also diagnosed with hearing impairments, one with renal tubular acidosis and one with bilateral ureterovesical refluxes.

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P12.006
X-linked Alport syndrome investigation in Hellenic families. G624D mutation in *COL4A5* may explain many familial hematuria cases in Greek mainland that hardly can be diagnosed as Alport syndrome

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The X-linked Alport syndrome (ATS) is caused by mutations in *COL4A5*. ATS is heralded with continuous micro-hematuria which rapidly progresses to proteinuria, hypertension and chronic or end-stage renal disease (ESRD) by adolescence, frequently accompanied by sensorineural deafness and ocular complications. Milder forms of ATS also exist. We initially studied nine Hellenic families suspected clinically of X-linked ATS who presented with marked phenotypic heterogeneity. We identified four mutations in *COL4A5* in six families.

Males currying E228X or c.2946delT mutation had the classical ATS symptoms with early onset of renal failure and deafness. However four males with the milder mutation G624D, belonging in two families originating from Greek mainland, reached ESRD after 39-yo and one patient showed Thin Basement Membrane Nephropathy (TBMN: a milder phenotype than ATS, classically attributed to *COL4A3/COL4A4* genes). Another 5/8 affected males with mutation P628L also developed ESRD between 30-57-yo, while three exhibit only mild chronic renal failure (CRF). Surprisingly, screening three more hematuric families from Greek mainland for G624D, were found positive for this mutation. The data support previous findings that certain mutations are associated with milder phenotypes, such as TBMN and familial microscopic hematuria. G624D may explain a lot of familial hematuria cases in Greece. We are designing a Greek genetic epidemiological study in order to find the exact frequency of the mutation in hematuric patients. Similar conclusions apply for missense mutation P628L. Interestingly, mutations G624D and P628L are near the 12th natural interruption of *COL4A5* triple-helical domain, which may explain the milder phenotype.

P12.007
Three novel mutations in Turkish patients with Alstrom Syndrome

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Alström syndrome (AS) is a rare multisystemic disorder. Major clinical symptoms of the disease are congenital retinal dystrophy leading to blindness, neurosensorial hearing lost, childhood obesity, insulin resistance, and type 2 diabetes, dilated cardiomyopathy. Mutant form of *ALMS1* gene causes AS. *ALMS1* is a novel gene which is comprised of 23 exons encoding a protein of 4169 amino acids that does not share significant sequence homology with any other known genes. The ALMS1 protein is ubiquitously expressed and localizes to centrosomes and basal bodies of ciliated cells, perhaps playing an important role in cilia function and intraflagellar transport. RNA interference knockdown experiments indicate that a total lack of ALMS1 impairs cilia formation. To date, more than 100 mutations, mostly nonsense and frameshift type, in *ALMS1* have been reported in Alström syndrome. The reported mutations are primarily clustered in exons 16, 10, and 8, but less common mutations also occur in exons 12, 18 and 11. In present study, four Turkish AS patients were analysed. Mutation screening of the whole coding region and all intron/exon boundaries were performed by PCR and direct sequencing. As a result of sequence analysis, three novel mutations; c.4155insA; T1386NfsX15, c.5311C>T; p.Q1771X, c.9749C>A; p.S3250X and several polymorphism; IVS4+20T>A, F731F, V673G, D2674H, R4031K, IVS18-12T>C were detected in patients. This study was supported by State Planning Organization of Turkey (Project No: DPT 2006 K120640)

P12.008
APP gene mutation (D678N) in a patient with an Early-Onset Alzheimer‘s Disease

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Introduction: Notably, the clinical presentation of familial forms of AD (FAD) is more complex, and mutations of APP gene have also been described in these patients. Only about 26 different missense mutations, located in exons 16 and 17 of APP, have been reported. Aims: To better assess the genetic contribution of APP to FAD, we performed a systematic mutation analysis of this gene in a series of Italian patients with FAD. Methods: Ninety-seven patients (age, 66.48 ± 10.1 years; mean ± SD) with FAD were recruited at the Institute of Neurology, University “Magna Graecia” in Catanzaro, and subsequently screened for mutations at the Institute of Neurological Sciences, National Research Council, in Cosenza, Italy. The exonic regions of APP (exons 16 and 17) gene were amplified and a mutational screening was done by DHPLC and direct sequencing. Results: Within our FAD, a non-synonymous change in exon 16, previously described in a Japanese pedigree, was detected (D678N) in a proband with cognitive decline having had commenced at 58 years of age. We found five affected subjects of the same family, who showed cognitive disorders with the age at onset ranging from 50 to 65 years. Conclusion: In this study, we found a D678N mutation in a patient showing a inheritance pattern consistent with EO-autosomal dominant transmission. Our data confirmed that APP is actually an EO-AD gene, in which onset occurs primarily prior to 65 years of age and often much younger; and for a given mutation, onset ages are tightly clustered.

P12.009
Missense mutations in myosin light chain kinase MYLK in familial thoracic aortic aneurysms and dissections (TAAD)

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Background: A recent study showed that mutations in myosin light chain kinase MYLK may cause familial thoracic aortic aneurysms and dissections (TAAD). Five mutations were found in 193 probands.
Two previously identied TAAD genes, MYH11 en ACTA2, encode respectively the SMC-specific isoform of actin and myosin heavy chain, while MYLK encodes the MCLK protein. This kinase phosphorylates regulatory light chain (RLC), a protein that regulates the actin and myosin activities and actin-myosin II cytoskeleton-mediated functions inside the smooth muscle cells of aortic wall. These processes are important in maintaining the aortic wall, which is subject to biomechanical forces throughout life.
Methods: We studied the MYLK gene in 95 probands from TAAD families by high resolution melting curve analysis, followed by sequencing of aberrant fragments. All patients had negative test results for sequencing of the genes FBN1, TGFBR1, TGFBR2, MYH11 and ACTA2.
Results: Two missense mutations were identified in the MYLK gene: c.2183G>A (p.Arg728His) and c.4565C>T (p.Val1522Ala). Both mutations were not detected in 120 ethnically matched healthy controls. The mutations involve highly conserved amino acids, 100% conserved down to Tetraodon, in highly conserved domains. However, there is only a small difference in properties of the variant amino acids.
Conclusion: We identified 2 missense mutations in 95 (2.1%) families with TAAD. This finding is in agreement with the result of the previous study. However, to assess clinical significance of the identified mutations, further studies, such as functional analysis of missense mutations, are needed.

P12.010
Arthrogryposis multiplex congenita associated with a heterozygous mutation and 855 kb microduplication in 17q12 both likely affecting function of the cation channel gene ACCN1 in a male fetus.

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A very severe form of congenital arthrogryposis multiplex was identified by prenatal sonography in the 22th week in the second pregnancy of a 25 years old healthy woman of Austrian origin. Her 4 years old son is healthy as is her non-consanguineous 28 years old husband, also of Austrian extraction. Because of an elevated risk (1:38) for fetal Down syndrome based on combined test results, an amniocentesis in the 16th week of an otherwise thus far uneventful pregnancy was performed, revealing normal cytogenetic and biochemical (AFP) results. However during midtrimester targeted fetal organ screening in the 22th week a complete absence of fetal movements (akinesia) and further fetal anomalies were recognized, leading to the diagnosis of congenital arthrogryposis multiplex with some similiarities to Pena-Shokeir-syndrome, a heterogeneous autosomal recessive disease. Because of the bad prognosis parents decided to derminate the pregnancy and further genetic analyses as well as fetal autopsy were performed. A 60k Agilent array CGH analysis of fetal DNA showed a 855 kb microduplication of 17q12 in the fetus and his mother. Because one of the chromosomal breakpoints is quite likely to affect the ACCN1 gene, we consecutively performed DNA sequencing of this gene from fetal DNA. The heterozygous missense mutation c.137G>T[p.R46L] was identified, and this rare mutation was found to be of paternal

origin. Although Pena-Shokeir-syndrome and related disorders are heterogeneous and several potential causative gene have been identified so far, we seriously consider the compound heterozygous mutations described here as another potential pathogenetic mechanism in such disorders.

P12.011
LRRK2 haplotype analysis in exon 41 p.G2019S positive patients with Parkinson disease.

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Mutations in the *LRRK2* gene have been found to be responsible for autosomal dominant forms of Parkinson Disease (PD).
The most common *LRRK2* p.G2019S mutation, laying in exon 41, has been reported to account for 5-6% of familial PD and 1.6% of sporadic PD in Europe. Three different *LRRK2* haplotypes have been associated with this point mutation: haplotype 1, characteristic of North African, Arabs and western European subjects; haplotype 2, typical of American European-descent individuals; haplotype 3, identified in Japanese families. The p.G2019S mutation seems to be occurred independently at least twice in humans: once on the haplotype 1 and once in the phylogenetically related haplotype 2 and 3.
A total of 2261 unrelated consecutive PD patients (Parkinson Institute Biobank of Milan - http://parkinson.it/dnabank.htlm) has been screened and 35 subjects were found to carry exon 41 *LRRK2* variants:
30 p.Gly2019Ser heterozygotes (of which one was also heterozygote carrier of IVS41+30 A>G)
1 p.Gly2019Ser homozygote
1 p.Ile2020Leu heterozygote
1 p.Pro2036Arg heterozygote
1 p.Tyr2018Tyr heterozygote
1 p.Pro2007Pro heterozygote
In addition, 5 subjects were found to be carrier of intronic variants (2 IVS40-39 A>G heterozygotes, 1 IVS40-103 T>C heterozygote; 1 IVS41+30 A>G heterozygote; 1 IVS40-47 A>G heterozygote).
Ten *LRRK2* SNP markers, spanning from exon 8 to 43, have been directly sequenced in the 31 p.G2019S carriers. According to previous studies we found that the haplotype 1 is the most frequent in p.G2019S carriers in our population suggesting that the mutated allele derived from a single common founder probably of North-African origin.

P12.012
Alpha-synuclein gene rearrangements in autosomal dominant form of Parkinson's disease in Russia.

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Recent studies have shown that the development of Parkinson's disease (PD) may be affected by the increase in the alpha-synuclein gene (*SNCA*) copy number (duplications and triplications). We have assessed the frequency of *SNCA* rearrangements in 39 patients (average age 58.4±10.4, average age at disease onset 44.4±11.9) with autosomal dominant PD form. Alpha-synuclein gene dosage analysis was carried out using Real-time PCR on an ABI 7000 genetic analyzer (Applied Biosystems) with Taqman probes. Beta-globin gene was amplified in one tube with exon 3 of *SNCA* as an internal control. All samples were tested in triplicates. To assess the *SNCA* gene copy number we compared the values of the threshold cycle of alpha-synuclein with those of beta-globin. Ratios from 0.8 to 1.2 indicated a normal gene dosage; from 1.3 to 1.7 - a heterozygous duplication; and from 1.8 to 2.2 - a triplication or a homozygous duplication. For all samples we obtained values which were in the range 0.8 to 1.2. These results did not reveal multiplications of the *SNCA* gene in the examined patients and allowed to suggest that *SNCA* duplications and triplications are a rare cause of autosomal dominant PD in Russia.

P12.013
Association study between the LINGO1 gene and Parkinson's disease in the Italian population

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A genome wide association study in European and American patients with Essential Tremor(TE)showed a significant association with the rs9652490 SNP of the leucine rich repeat and Ig domain containing 1 (LINGO1) gene. LINGO1 gene codes for a nervous system-specific transmembrane protein. Its expression is increased in the substantia nigra of some PD patients, thus suggesting that LINGO1 may be involved in PD pathophysiology. In this study, we performed a case-control analysis in an Italian population to assess the role of the LINGO1 gene in PD patients.
A total of 567 patients with PD and 468 control subjects were enrolled in Central-Southern Italy. Genotyping of LINGO1 SNPs (rs9652490 and rs11856808) was performed by TaqMan pre-designed assays using an ABI 7900 HT-SDS system.
No significant differences were found in allele or genotype frequency distribution of rs9652490 between patients and controls (P>0.05). Regarding rs11856808, the C allele of this marker was more frequent in patients than in controls, but after Bonferroni correction only a borderline P-value was obtained from the comparison (P=0.024; Padjusted=0.048). Genotype frequencies did not differ between the two groups.
Our study demonstrates that LINGO1 SNPs are not associated with our PD patients, thus showing that this gene does not represent a possible risk factor for PD in our population.

P12.014
A comprehensive mutation analysis of the PINK1 gene in Southern Italian patients with early- and late-onset parkinsonism

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Mutations in the PINK1 gene are associated with both familial recessive and sporadic early-onset Parkinson's disease (EOPD).
Data on Italian EOPD patients show mutations in a relatively high percentage (8-9%). Heterozygous or compound heterozygous mutations were also reported in a small number of late-onset PD patients (LOPD). In this study, we aimed at assessing the frequency and possibly the pathogenic role of PINK1 mutations in familial and sporadic patients with EOPD and LOPD coming from Southern Italy.
We selected 115 patients, including 102 cases with EOPD, 9 of which had a positive family history, and 13 familial cases with LOPD. All the eight PINK1 exons were analyzed by PCR and sequencing. The MLPA method was used in the heterozygous patients in order to detect exon dosage changes, caused by genomic rearrangements, of the known PD genes. Four already know different mutations (three homozygous: Q126P, W437X , Q456X and one heterozygous: E476K) and one novel heterozygous mutation (R207Q) were found in five patients (4.3%). In particular, 4 out of 103 EOPD patients (3.9%) carried PINK1 mutations. This frequency is lower than previous studies on Italian EOPD have suggested. Furthermore, a frequency of 1 out 13 (7.7%) in familial LOPD patient was found, thus confirming a certain influence of the PINK1 gene also in LOPD as observed in other studies. In conclusion, our study confirms the role played by the PINK1 gene mutations both in heterozygous and homozygous condition in PD development.

P12.015

CAA interrupted SCA2 alleles of 38 repeats jointly several SCA8 expansions segregate from a late onset parkinsonism index case

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Several Spinocerebellar Ataxia genes (SCAs) *SCAs1-3*, *SCAs6-8, SCA12* and SCA17 have been cloned, generally sharing a CAG (CAG/CTG in SCA8) repeat expansion mutations which usually encodes a polyglutamine tract. In our Spanish casuistic, SCA2 was found in 45 index cases over 408 familial cases with ataxia (25.47 % ± 3.90). No SCA mutation was found in any of 1,089 sporadic and idiopathic cases. Is generally known that SCA2 expanded alleles without CAA interruptions are associated with pathological development of the disease and that the interruptions would prevent elongation of the expanded sequence. In this study we present one pedigree showing SCA2 expanded alleles with CAA interruptions coexisting with SCA8 expanded alleles in several members. The index and founder case shows a SCA2 interrupted expanded allele of 38 CAG repeats coexisting with an SCA8 allele of 89 repeats. He presents late onset essential tremor and cerebellar atrophy. No mutations were found in *LRRK2* and *Parkin* genes associated with Parkinson's disease as his aetiology. One of his sons and two of his grandchild's share the same expanded interrupted SCA2 allele, coexisting with SCA8 expansions from 78 to 84 repeats, and all of them remain asymptomatic. In conclusion, SCA2 expanded alleles in pathological range with CAA interruptions could be associated with late onset Parkinsonism with uncertain penetrance. The role of SCA8 expansion coexisting with SCA2 expanded alleles remains unknown.

P12.016**

Compensatory molecular and functional mechanisms in neurons of the *Grm1*^{crv4} mouse, a murine model for ataxia lacking the mGlu1 receptor.

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The metabotropic glutamate type 1 (mGlu1) and 5 (mGlu5) receptors are the only members of group I mGlu receptors, a subfamily of G protein-coupled receptors implicated in synaptic plasticity, and in mechanisms of feed-back control of glutamate release. mGlu1 and mGlu5 receptors exhibit a complementary distribution throughout the central nervous system, more evident in the cerebellum, where mGlu1 receptor is most intensely expressed while the mGlu5 receptor is not. Despite the different distribution, they show a similar subcellular localization relative to transmitter release sites, and use common transducing pathways.

We recently described the *Grm1*^{crv4} mouse mutant carrying a spontaneous inactivating mutation of the mGlu1 receptor. Homozygous *Grm1*$^{crv4/crv4}$ mice exhibit a complex phenotype with ataxia. To better define the pathophysiological mechanisms of ataxia in these mice, we evaluated expression and function of the other member of group I mGlus, the mGlu5 receptor, in *Grm1*^{crv4} mouse cerebellar and brain cortices. Western blot and immunofluorescence analyses show mGlu5 receptor overexpression in the homozygous *Grm1*$^{crv4/crv4}$ mice. Real-time quantitative RT-PCR results indicate that up-regulation is already evident at RNA level, suggesting a cellular compensatory mechanism due to the absence of active mGlu1 receptor molecules. Finally, functional studies confirm an enhanced glutamate release from cortical cerebellar and brain synaptosomes as respect to wild type, that is abolished by mGlu5 receptor specific inhibitors.

Mechanisms that affect glutamate bioavailability and glutamatergic transmission at the synaptic level may contribute to the development and/or to the rescue of the affected phenotype.

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P12.017

Prodynorphin (PDYN) mutations are rare in Spanish patients with Spinocerebelar Ataxia

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Spinocerebellar ataxias (SCAs) represent a group of dominantly inherited neurological disorders characterized by variable degeneration in the cerebellum, spinocerebellar tracts, and brain stem. The SCAs present with gait ataxia, dysarthria, oculomotor abnormalities and may have additional symptoms. Recently, missense mutations in prodynorphin (PDYN), the precursor protein for several opioid neuropeptides have been reported as the cause of SCA23. In order to investigate the contribution of this new SCA gene in Spanish patients we studied 335 individuals with clinical diagnosis of SCA and negative for SCA 1, 2, 3, 6, 7, 12, 17 and DRPLA. A positive family history was present in 43% of cases, 28% were sporadic and for the reminding cases family history was unclear or unknown. All exons and exon-intron boundaries of PDYN (NM_024411.4) were sequenced on an ABI3730 analyzer and sequence analysis was performed with the Staden package. All identified sequence variations were screened in 186 control individuals without neurological disorders. We identified four sequence changes in non-coding exons 1 and 2 (c.-241 G>C, c.-162 A>G, c.-124 G>T and c.-48 G>C). In addition, a synonymous alteration (c.72 G>A; p.Ser24Ser) was identified in exon 3 and a single nucleotide substitution c.130-46 T>G in intron 3 of PDYN. None of these changes was present in searchable databases of human polymorphisms and they were not detected in healthy controls from our population. SCA 23 appears to be a very infrequent cause of SCA in Spain. Whether these new sequence variations in PDYN have any functional effect needs further investigation.

P12.018

Genetic and epidemiological data of spinocerebellar ataxia type 8 in Spanish population

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Autosomal Dominant Cerebellar Ataxias (ADCAs) are a clinically and genetically heterogeneous group of neurodegenerative disorders in which several Spino-Cerebellar Ataxia genes (SCAs) have been cloned: *SCA1-3, SCA5-8, SCA10-11, SCA13-15, SCA27-28* and *SCA31*. We have analyzed 408 unrelated familial and 1,089 sporadic and idiopathic cases of SCA. Over the familial cases 6.25% cases were SCA1; 25.57% SCA2; 34.09% SCA3; 7.39% SCA6; 5.68% SCA7; 14.77% SCA8; 3.41% SCA17; and 2.84% DRPLA. About 55% of our pedigrees remained genetically unclassified and no SCA mutations were detected in the 1,089 isolated and idiopathic cases. In 26 pedigrees with SCA8 expansions the allele size ranges from 85 to 726 repeats (140.94 ± 105.90; Pearson Coefficient= 75.14%). Maternal transmissions presented elongations of the CAG/CTG combined sequence ranging from +3 to +13 repeats. In contrast paternal transmissions presented contractions from -1 to -17 repeats. Giant SCA8 expansions ranges from 270 to 1,126 (N=13), all carried by unaffected adult individuals. These expansions have been originated from homozygous SCA8 females with alleles of moderate size in two unrelated pedigrees. Remarkably, homozygous males have transmitted contracted alleles, as in heterozygous cases occurs. The distribution of SCA8 alleles in a sample of 90 individuals from general population is bimodal, with two groups: 15 to 34 CAG/CTGs with frequency of 98% and 77 to 86 with frequency of 2%. SCA8 alleles with moderate size in general population could be the origin of new expanded alleles.

P12.019***

Prodynorphin mutations cause the neurodegenerative disorder spinocerebellar ataxia type 23

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Spinocerebellar ataxias (SCAs) are dominantly inherited neurodegenerative disorders characterized by progressive cerebellar ataxia and dysarthria. We have identified missense mutations in the prodynorphin (*PDYN*) gene to cause SCA23 in four Dutch ataxia families. PDYN is the precursor protein for the opioid neuropeptides, α-neoendorphin, and dynorphins A and B (Dyn A and B). PDYN peptides regulate pain processing and modulate the rewarding effects of addictive substances. Three mutations are located in Dyn A, a peptide with opioid activities, as well as non-opioid neurodegenerative actions, while a fourth mutation is in the non-opioid PDYN domain. The mutations stabilize Dyn A by restricting its conversion to shorter enkephalins. In addition, two of the mutant Dyn A peptides induced toxicity above that of wild type Dyn A in cultured striatal neurons. The mutation in the non-opioid domain was associated with altered expression of components of the opioid and glutamate system as evident from analysis of SCA23 autopsy tissue. In line with our findings, Dyn A was already found to be upregulated in aging rodent brain contributing to age-related impairment of spatial learning and memory and additionally in the prefrontal cortex of Alzheimer disease patients, elevated Dyn A levels correlated with neuritic plaque density. Thus, alterations in Dyn A activities and/or impairment of secretory pathways by mutant PDYN may lead to glutamate neurotoxicity which underlies Purkinje cell degeneration and ataxia.

P12.020

Investigation of correlation between triplet repeat numbers in ATXN1, ATXN2, MJD, CACNA1A, ATXN7, ATXN8OS, ATXN10, PPP2R2B, TBP genes and age of onset in SCA suspected patients

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Introduction: Spinocerebellar ataxias (SCAs) are a group of neurogenetic diseases caused by expansion of triplet repeats in various genes on different chromosomes. SCA1, 2, 3, 6, 7, 12 and 17 are produced by CAG repeats and SCA8 and10 by CTG and ATTCT repeats, respectively. Expanded repeats in SCA1, 2, 3, 6, 7 and 17 and the SCA8 coding strand code for polyglutamine tracts. Previous studies have shown variable results concerning the correlation between numbers of triplet repeats in these genes and the age of onset in patients.

Materials and Methods: 52 patients with clinical features resembling SCA were analysed. DNA was extracted and PCR performed for the region of the genes containing the triplet repeat. The PCR products were electrophoresed in polyacrylamide gels. repeat numbers were scored and the data analysed using SPSS software.

Results: There was no significant correlation between triplet repeat numbers in two alleles of all genes analysed and age of onset in all SCA suspected patients. A non-significant correlation was observed between the number of triplet repeats in the *ATXN7* and *ATXN8OS* genes and age at onset. In other genes a non-significant inverse correlation was observed.

Discussion: Our hypothesis was that triplet repeat numbers in some of the analysed genes and age of onset would be significantly correlated, but our results did not support this. We conclude that in the Iranian population, expansion in triplet numbers does not correlate with age of onset. However, the small number of patients studied or errors in the remembered age of onset could also cause this result.

P12.021

Polyglutamine-expanded protein ataxin-3 induces mitochondrial ERK activation and autophagic cell stress

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Spinocerebellar ataxia type 3 (SCA3), also called Machado-Joseph disease, an autosomal dominant neurodegenerative disease, is caused by an increased number of CAG trinuclear expansion repeats in coding regain of *Ataxin3* and resulted in a disease protein with a higher polyQ domain. Although the detail mechanism of pathogenesis is yet to be defined, neurotoxin, especially reactive oxygen species (ROS), released from aggregated mutant proteins, may play a role in the pathogenic process. Previous studies also suggested that mitochondrial phosphorylated extracellular signal-regulated protein kinase1/2 (p-ERK1/2) was induced oxidative stress and lead to cell death. In this study, two lymphoblastoid cell lines were isolated from SCA3 patients, one contains 31 and 82 CAG repeats in ataxin-3 individual, denoted MJD07; another contains 30 and 78 CAG repeats, denoted MJD08. We investigated the crucial relationship between the expression of p-ERK1/2 and autophagy. Results found the endogenous p-ERK1/2 and autophagy marker protein, Atg8 (LC3 class II) were higher in SCA3 patients. Electron micrographs showed that only the cells expressing expanded ataxin-3 contained aggregated protein and autophagic vacuoles. Based on the above observations we hypothesized that the aggregated mutant Ataxin-3 proteins may generate ROS in mitochondria, which subsequently up-regulate p-ERK1/2 and Atg8 expression levels and ultimately lead to autophagy and cell death.

P12.022

High incidence of DYNC2H1 mutations in a cohort of 51 patients with ATD/SRPIII

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The short-rib polydactyly (SRP) group includes 4 lethal disorders i.e. type I Saldino-Noonan, type II Majewski, type III Verma-Naumoff, and type IV Beemer-Langer and 2 disorders compatible with life, namely asphyxiating thoracic dysplasia (ATD) and Ellis-van Creveld (EVC) syndrome. They are characterized by autosomal recessive inheritance, short ribs, short long bones, inconstant polydactyly and trident acetabular roof but distinct by variable malformations and metaphyseal changes. Recently, 6 genes encoding primary cilia proteins have been involved in the SRP group (EVC/EVC2, IFT80, DYNC2H1, NEK1 and TTC21B), confirming that this group belongs to the ciliopathy spectrum. We have also demonstrated that SRPIII and ATD belong to the same heterogeneous spectrum of conditions caused either by IFT80 or by DYNC2H1 mutations.

The aim of our study was to screen DYNC2H1 and IFT80 in 51 ATD/SRPIII consanguineous families or with sib recurrence and to identify other disease genes.

We identified no mutation in IFT80 but DYNC2H1 mutations in 16 cases and the screening is still in progress for 13 cases. Among 24 consanguineous families unlinked to DYNC2H1 or IFT80, a genome wide search was performed in 9 and led to the identification of 3 regions of homozygosity respectively for 3 families originating from Algeria, Turkey and Yemen. In each region of interest, candidate genes selected from the cilia database are currently tested.

We conclude that DYNC2H1 is involved in a significant part of ATD-SRPIII spectrum. Ongoing studies will hopefully lead to identify other disease genes presumably also involved in primary cilia function.

P12.023
Deafness due to mutations in *Otoferlin* gene (*OTOF*): additional identification of so far unreported mutations and unclassified variants

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Background: Auditory neuropathy spectrum disorders (ANSD) are characterised by preserved outer hair cells function (normal otoacoustic emissions and/or cochlear microphonic potentials) but inner hair cells and/or cochlear nerve disrupted function (abnormal auditory brainstem evoked potentials). ANSD may be caused by OTOF gene mutations (DFNB9)(so far 36 genes cause recessive form). We screened for OTOF gene mutations in belgian patients with ANSD.

Methods: Patients' DNA was studied after family history, clinical evaluation were completed (absence for syndromic form, thyroid dysfunction, heart rhythm, kidney or inner ear malformation; mutations in GJB2, GJB6 genes were excluded). Molecular investigation on DNA double-strand direct sequencing of the 46 coding exons of OTOF gene (NM_194248.2, long isoform, 1997 aa)(Dye Terminator Sequencing v3.1/ABI 3130xl) was performed. Exons 19-46 of the long isoform correspond to exons 1-28 of the short isoform (NM_004802.3, 1230 aa). Unclassified variants (UV) refer to changes absent from Ensembl and Uniprot databases or from publications.

Results: 10 unrelated patients, non consanguineous pedigree were included. None had temperature-sensitive auditory neuropathy. One patient was identified homozygous for the reported c.3269C>A mutations; one for compound heterozygous c.2401G>T and c.2402A>T mutations as well as UV (c.3608A>G). Four patients were identified with UV (one patient: c.2123G>A /c.2406+9G>A) and 1 UV (three patients with UV: p.Asn727Ser, p.Cys1251Gly, p.Arg1853Gln). 4 patients did not show any substitution. Parental screening is pending.

Conclusions

These results underline OTOF gene structure complexity (numerous mRNA isoforms and UV). Once identified, screening in parents/relatives and gene expression studies are needed to better understand their possibly pathogenic role.

P12.024
Mutations in *PYCR1* gene in three families with Autosomal Recessive Cutis Laxa, Type 2

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Autosomal recessive cutis laxa, type 2 (ARCL2), is a rare disorder of connective tissue in which the skin sags excessively, giving to the individual an aged aspect. It has been suggested that ARCL2 is the same entity described under the denominations of wrinkly skin syndrome and gerodermia osteodysplastica. Such confusion is caused, in part, by overlapping of the histological and clinical findings, thus nosology remains uncertain until the definitive elucidation of the molecular basis of these conditions. In the present study we analyzed three unrelated families who presented clinically with ARCL2 for mutations in three genes implicated in several forms of cutis laxa; *ATP6V0A2*, *SCYL1BP1*, and *PYCR1*. No causative mutations were identified in *ATP6V0A2* and *SCYL1BP1* genes. However, screening for mutations in *PYCR1* revealed two new variant by direct DNA sequencing. Patients 1 and 2 were both homozygous for a missense alteration replacing the triplet CAG of the codon 10 by TAG, creating a stop codon that determined the loss of 298 codons. Patient 3 showed a homozygous missense alteration (c.722C>T; Ala241Val), however it will be necessary confirm if this variant is present or not in a large cohort of control individuals. It is important highlight that *PYCR1* gene performs a critical role in proline biosynthesis. The genetic changes found in this work contribute to the elucidation of the molecular basis of cutis laxa. Further studies should be conducted in individuals with other phenotypic presentation to establish the nosology of this group.

P12.025
In Search of Oligogenicity in Bardet-Biedl Syndrome

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Bardet-Biedl syndrome (BBS) is a model disease for ciliopathy in humans. The remarkable genetic heterogeneity that characterizes this disease is consistent with accumulating data on the interaction between the proteins encoded by the 14 BBS genes identified to date. Previous reports suggested that such interaction may also extend to instances of oligogenic inheritance that defies the long held view of BBS as an autosomal recessive disease. In order to investigate the magnitude of oligogenic inheritance in BBS, we conducted a comprehensive analysis of all 14 BBS genes as well as the *MGC1203* modifier gene in a cohort of 29 BBS families, most of which are multiplex. Two in *trans* mutations in a BBS gene were identified in each of these families for a total of 20 mutations including 12 that are novel. In no instance did we observe two mutations in unaffected members of a given family or observe the presence of a third allele that convincingly acted as a modifier of penetrance or even expressivity. In addition to presenting a comprehensive genotype/phenotype overview of a large set of BBS mutations, including the occurrence of nonsyndromic retinitis pigmentosa in a family with a novel *BBS9* mutation, our study strongly argues against the oligogenic model of BBS in the overwhelming majority of cases.

P12.026
Screening Iranian thalassemia intermedia patients with normal or carrier status of beta-globin gene for mutations in 5'HS3 and 5'HS4 LCR core regions and NF-E2

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Analysing beta globin gene sequence in 114 Iranian individuals with thalassemia intermedia phenotype revealed homozygous or compound heterozygous beta-globin mutations to be the major primary disease factor in 86.2% of cases. However, 8.2% of these individuals were found to be heterozygous or wild type for beta-globin mutations. In search for determinants outside of the beta-globin gene, which could be responsible for the unexpected thalassemia intermedia phenotype in these subjects, we screened the alpha-globin genes, the 5'HS3 and 5'HS4 regions of the beta-globin LCR and the NF-E2 transcription factor for sequence variations in selected individuals. The -3.7 deletion was the only alpha-globin mutation detected, and no alterations were found in 5'HS3 and NF-E2. Sequence analysis of the 5'HS4 LCR core region identified three known SNPs in a single patient, who required irregular blood transfusions. The A/G polymorphism in the 5'HS4 palindromic region was also observed to be variable. Family studies were carried out on a female G/G homozygous patient , who received irregular blood transfusions. Her father, who had the same heterozygous IVSII-1 beta-globin mutation, but the A/G genotype at the 5'HS4 palindromic site, presented with mild anemia and no requirement for blood transfusions. This suggests an impact of SNPs in the 5'HS4 LCR core region on the thalassemia phenotype, and offers an interesting subject for further investigations in the Iranian population.

P12.027
Notch 1 mutations in patients with aortic aneurysm and bicuspidal valve

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NOTCH1 encodes for a transmembrane protein that activates a signaling pathway with an active role in cardiac embryogenesis, including aortic and pulmonary valve development.We, therefore, investigated the potential relationship between mutations in regions of *NOTCH1* recently reported to be associated with bicuspid aortic valve. We performed a sequencing analysis of 9 of coding exons (exons 10, 11, 12, 13, 20, 23, 29, 30, 34) of NOTCH1 gene in 47 patients with sporadic BAV. Our analyses revealed 17 NOTCH1 heterozygous variants in the analyzed patients. Nine are located within exons and 8 within intronic. Seven variants were described previously as polymorphisms. However, five coding substitutions and one silent were neither listed in public SNP databases nor in the literature and were therefore considered novel. Two novel nucleotide changes, one missense mutation c.3679 C > T located in exon 23 and one polymorphism c.7347 C > A located in exon 34, were nonsynonymous leading to amino acid substitutions (p.P1227S, p.S2449R, respectively). Out of five patients, one had bicuspidal valve and aortic aneurysm, two - aortic aneurysm and valve calcification and two bicuspidal valve, aortic aneurysm and valve calcification.

Four unique, nonsynonymous (2 novel) variants were identified in 4 (8,5%) of 47 patients with concomitant bicuspid aortic valves).

The principal finding of this targeted mutation analysis of the *NOTCH1* gene is the identification of one novel missense mutation among patients with BAV phenotype. The studies warrant further investigation into the role of *NOTCH1* missense variants in the pathogenesis of aneurysmal associated valve disease.

P12.028
A novel autosomal recessive cardiac and brain malformation syndrome in a consanguineous Indian family

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Two siblings of healthy consanguineous Indian parents were born with congenital heart and brain malformations including double outlet right ventricular, bilateral frontal polymicrogyria, atrophy and periventricular cysts, agenesis of the corpus callosum, and atypical Dandy-Walker malformation. Both children (a brother and sister) died by 3 months of age. Routine blood chromosome analysis, FISH for DiGeorge syndrome and 1 Mb-BAC chromosomal microarray analysis (CMA) were all normal. However, CMA-SNP analysis identified a 7 Mb region of absence of heterozygosity (AOH) at chromosome 7p14 shared between these two affected siblings. This region contains 46 annotated genes, one of which (COBL) was identified as a strong candidate gene. Cordon-bleu (COBL) is a conserved and novel actin nucleation ciliary gene involved in neural tube formation in mouse and chick embryos. Direct sequencing and expression studies of COBL are pending.

P12.029
Gene expression profiling reveals novel insights into the molecular pathogenesis of brittle cornea syndrome and Ehlers-Danlos syndrome kyphoscoliotic type

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Brittle cornea syndrome (BCS) is a rare autosomal recessive connective tissue disorder characterized by thin, fragile cornea leading to corneal rupture upon minor trauma, blue sclerae and joint and skin hyperlaxity. The condition is caused by mutations in *ZNF469*, encoding a Zinc Finger 469 protein of unknown function which belongs to the C_2H_2-zinc finger protein family. Clinical comparison reveals considerable overlap with Ehlers-Danlos syndrome kyphoscoliotic type (EDS VIA), caused by mutations in *PLOD1*. We performed direct sequencing of *ZNF469* in two consanguineous Turkish BCS families and identified novel homozygous *ZNF469* mutations, respectively c.6509_6512dupTCTT (p.Leu2171PhefsX99) in F1 and c.9792dupT (p.Ala3265CysfsX6) in F2. To unravel the molecular pathogenesis of BCS and the relationship with EDS type VIA, we studied gene expression profiles on the Affymetrix Human Gene 1.0 ST platform using patient's fibroblasts with either a homozygous frameshift (n=2) or missense (n=1) mutation in *ZNF469* alongside EDS VIA fibroblasts carrying *PLOD1* mutations (n=3). Comparison with wild type controls (n=6) identified deregulation of *COL8A1*, *PLOD2*, *LOXL2*, *FBN2* and *MMP12*, next to other extracellular matrix related genes, in both BCS and EDS VIA fibroblasts. Deregulation of several players of the Wnt-pathway, important in ocular embryogenesis, was also observed in both BCS and EDS VIA fibroblasts and implicate this developmental pathway in the pathogenesis of these connective tissue diseases. These novel findings suggest a role for the ZNF469 protein in connective tissue homeostasis and ocular development and provide important new insights into molecular pathways involved in EDS and related connective tissue diseases.

P12.030
A novel Brugada syndrome variant shown to be causal based on segregation analysis and familial genotypic-phenotypic correlation.

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Brugada syndrome is an autosomal dominant inherited disorder characterised by a primary cardiac arrhythmia in a structurally normal heart, with a propensity to sudden cardiac death. Genetic defects have mainly been attributed to mutations in the SCN5A gene, coding for the alpha subunit of the sodium channel, although they account for only 20% of cases.

SCN5A mutation analysis of all 27 translated exons and flanking intron-exon boundaries in our outclinic patient population revealed a novel variant c.4300-2 A>T in intron 24. This variant was discovered in one of our clinically diagnosed Brugada syndrome index patients with a spontaneous typical type I electrocardiogram (ECG) and a positive family history of Brugada syndrome.

In silico splice prediction analysis corroborates the omission of splicing through abolishment of the splice acceptor site at the intron24-exon25 boundary, thereby affecting the formation of the DIII/S5-S6 of alpha-subunit of the sodium channel.

To further investigate whether this variant is causal for Brugada syndrome we tested all available 10 family members for the presence of this variant. The sequencing analysis demonstrated that the mutation segregates within the family according to spontaneous or drug-induced (ajmaline) type I ECG and not within the ajmaline negative and type I ECG negative family members.

Detection of this variant in a control population is ongoing to further prove its pathogenic character.

P12.031
Compound heterozygous mutations D1690N and G1748D in *SCN5A* gene associated with the Brugada Syndrome

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Purpose: Brugada Syndrome (BS) is an inherited cardiac disorder caused, in 20-25% of the cases, by mutations in *SCN5A* gene, which encodes for the alpha-subunit of the cardiac Na+ channel.

In this study, we identified two novel mutations in *SCN5A* in a BS patient.

Methods: To screen for mutations, the complete *SCN5A* coding region, including the intron/exon boundaries, was amplified by PCR and performed to direct sequencing. For each variant we made a prediction of the effect of mutation based on three online softwares

(Polyphen, PMUT and SIFT).
Results: Genetic analysis revealed that the proband carries two mutations, in exon 28, in two highly conserved amino-acids. The first one was a heterozygous mutation in c5068G>A, that causes the substitution D1690N in the linker between S5-S6 of the DIV of the channel. The second variant found was a G to A substitution at position c5243 that caused the mutation G1748D, which is located in the NH3 *termini* part of the segment S6 of the DIV of the channel.
Both mutations are predicted to affect protein function by the three softwares.
The familial study showed that the proband transmitted the G1748D mutation to a daughter. This result showed that the index case carried the mutations in separate alleles.
Conclusions: In this study, we described compound heterozygous mutations D1690N and G1748D in *SCN5A* associated with BS. The *in silico* analysis predicted that both mutations are pathogenic. Our data suggests an important role of compound heterozygosity in modulating the phenotypic expression of BS.

P12.032
Analysis of coding sequence of SNTA1 gene in Russian BrugadaSyndrome patients

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Background. Brugada syndrome (BrS) is inherited cardiac arrhythmic disorder characterized by ST-segment elevation in right precordial leads, conduction disorders and high risk of cardiac sudden death. The SCN5A gene was identified as causative in 1998 and has been only one known for BrS for many years, though mutations in this gene account 15%-30% of all cases. Starting from 2007 the list of genetic variants was increased up to 8 but all those genes seems to be much less prevalent and does not explain vast of majority BrS cases.
The aim of our study is to screen the coding sequence of *SNTA1*gene. SNTA1 protein is associated with dystrophin complex and SCN5A sodium channel.
Patients and Methods. We observed a cohort of 16 patients with clinical diagnosis “Brugada syndrome”. We did performed full coding analysis of SCN5A gene and did start screening of SNT1A coding sequence in SCN5A-negative BrS patients.
Results. Three BrS patients were carried SCN5A mutations (19% of cases). In SNT1A gene we did find one rare missence genetic variant c.R106Q in exon 2 in affected person. Two intronic substitutions c.IVS2+112g>a and c.IVS2+82g>t were found in three unrelated patients.
Conclusion. New rare genetic variants had been found in Russian BrS cohort (about 8%).To elucidate possible causative role of c.R106Q change in*SNTA1*gene further investigations are required. Population analysis of ethnically-matched controls and functional analysis of mutant protein will be performed.
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P12.033
Exploration of microRNAs that mis-target SCN5A as a new mechanism for Brugada Syndrome

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Brugada syndrome (BrS) is an autosomal dominant cardiac disorder associated with ventricular fibrillation and sudden death. About 20% of patients have mutations in SCN5A resulting in a loss of functional cardiac sodium channels. Rare mutations do exist in other genes, however, the genetic origin of BrS in the majority of patients remains unknown.
We aim to determine if rare variants in the SCN5A 3’UTR region can modulate channel expression by creating novel, (or by enhancing existing), targets of microRNAs. MicroRNAs are short single-stranded RNAs, which, when bound to specific targets in mRNA transcripts, cause post-transcriptional repression of protein expression. We sequenced the SCN5A 3’UTR (2384bp) in a cohort of 97 BrS patients without mutations in the SCN5A coding regions or other tested genes. Thirteen known SNPs and two new heterozygous variants were identified; c.*539A>G and c.*1677G>A, neither of which were detected in more than 150 controls.
In silico analysis of the two variants was performed using on-line programs predicting microRNA targeting to a given nucleotide sequence. Several microRNAs reported to be expressed in the heart were found to target the new variant 539, however we excluded the possibility that variant 1677 creates new target sites for microRNAs. We are pursuing the candidate microRNAs with in vitro studies.
It’s not known if SNPs in the 3’UTR can play a role in the regulation of SCN5A, however, we propose that these, or new variants, could cause or influence disease severity in BrS or other arrhythmias via the action of microRNAs.

P12.034
Hunting for novel dilated cardiomyopathy genes using haplotype sharing and exome sequencing

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Background: Idiopathic dilated cardiomyopathy (DCM) is a clinically highly heterogeneous disorder, characterized by dilation and impaired contraction of the left ventricle. Monogenic inheritance is observed in one-third of the idiopathic cases and mutations in the >40 known genes explain only ~25% of familial cases. In order to identify novel DCM disease genes, we investigated two DCM families by Haplotype Sharing Test (HST) combined with exome sequencing.
Methods: To localise novel DCM genes, 250K SNP genotyping was performed to identify regions shared between patients from a single pedigree. The HST was applied to (I) a family with 4 DCM and 2 peripartum cardiomyopathy patients and (II) a family with 3 DCM patients and 1 patient with reduced left ventricular function. To subsequently identify the corresponding disease genes, DNA of the index-patient and an affected relative from each family were analysed by exome sequencing.
Results: The HST revealed largest shared haplotypes of 71 cM on chromosome 15 containing ~600 genes (family I) and 46 cM on chromosome 9 containing ~475 genes (family II). Exome sequencing revealed potentially pathogenic variants shared by the two affected family members in both families. Confirmation by Sanger sequencing and carriership analysis in affected family members and healthy controls is in progress.
Conclusions: We identified potentially pathogenic variants in the largest shared haplotypes in two DCM families. Our results show that a combination of the HST and exome sequencing is a promising approach to identify novel genes underlying familial cardiomyopathies.

P12.035
Identification Of a Locus For Recessive Dilated-Cardiomyopathy On Chromosome 2 by Linkage Analysis in a Bedouin Family

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Cardiomyopaties are the most common disorders resulting in heart failure. Dilated cardiomyopathy (DCM), a disorder characterized by cardiac dilatation and reduced systolic function, is the most frequent cause. However, recessive neonatal isolated dilated cardiomyopathy has scarcely been associated with a mutation. We have identified patients with acute DCM in one tribe with consanguineous family of the Bedouin population. Three subfamilies contain five patients and four healthy siblings were investigated. The disease appears to be familial, with an autosomal recessive pattern of inheritance. SNPs array and then linkage analysis was performed using polymorphic microsatellite markers to confirm the linkage region on chromosome 2[2q35-2q36.3] with 10cM and to construct the haplotypes. The highest lod score for this region was 3.5 with two point analysis.

P12.036
Identification of a new chromosomal locus for a mutation causing Left ventricular non-compaction with ventricular tachycardia cardio-pathology.

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Structural and functional disorders of the heart are important causes of morbidity and mortality. Three Bedouin patients at ages 14-18 years of a single large consanguineous Bedouin family presented with Left ventricular non-compaction and sustained Ventricular Tachycardia. They were evaluated by Echocardiography which showed severe left side enlargement, severely depressed left ventricular (LV) function with focal lacunas in the LV free wall, but normal origin of the coronary artery. The 2 older patients were treated with recurrent electrical cardio version and intravenous administration of amiodarone and on discharge have undergone implantable cardioverter-defibrillator (ICD) implantation. The recessive pattern of inheritance in the consanguineous family suggested homozygosity of the mutation inherited from a common founder. We have performed homozygosity mapping using the affymetrix SNP 5 array on the patients and found a single large chromosomal block of homozygosity on chromosome 11q24 shared by all of them. Verification of this block with VNTR markers in all available family members proved linkage. The Lod score analysis, under the model of a recessive trait with full penetrance, using the PedTool server, was 2.46 for 2 point with marker D11S4958 and 4.59 for the multipoint analysis.
This chromosomal locus does not contain any of the known genes causing this disease; efforts are now being done to identify the mutated gene.

P12.037
New phenotype variance in the background of dynamin2 mutation

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Background: Dynamin-2 (DNM2) gene mutations cause two district human diseases: Charcot-Marie-Tooth disease (CMT) and centronuclear myopathy (CNM)). Here we report a 47 years-old women with severe cardiomypathy and centronuclear myopathy.
Methods: The patient underwent detailed neurological, cardiological, electrophysiological, myopathological and genetic sequence analysis.
Result: Left bundle branch block was first detected at age 40. The echocardiogram showed diffuse hypokinesis, with severe left ventricular dysfunction and dysyncrony. No coronary artery disease was detected and cardiac resyncronization device therapy was performed. The neurological symptoms started at age 42. Neurological investigation detected ptosis on the left side, strabismus convergent, myopatic face, atrophy and weakness in the small hand muscles, decreased tendon reflexes. The EMG found a myopathy and complex repetitive discharges. The exercise lactate test showed aerobe metabolic dysfunction. Muscle biopsy detected centronuclear myopathy. The DNM2 mutation analysis found a pathogenic heterozygous c. 1105 C>T (p. R369W) mutation in the exon 8 and 3 other polymorphisms.
Conclusion: It is the first case which reports about severe cardiomyopathy related to DNM2 gene mutations resulting in centronuclear myopathy. Our data broaden the phenotypic spectrum of the symptoms due to DNM2 mutations.

P12.038
MYBPC3-Glu258Lys related Hypertrophic Cardiomyopathy: a founder effect in Tuscany

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Background: MYBPC3 is the most common gene involved in HCM. Even if the majority of mutations identified are novel, it is possible to find some recurrent pathogenic mutation. A few founder mutations have been reported, and most of them are in the MYBPC3 gene. Interestingly, the occurrence of a founder effect has never been reported in Italy.
Objectives: The aim of the present study was to describe a founder effect in Tuscany caused by MYBPC3-Glu258Lys mutation.
Methods and Results: A total 401 Italian unrelated index HCM patients underwent screening for myofilament gene mutations by direct sequencing of 8 genes, including MYBPC3, MYH7, TNNI2, TNNI3, ACTC, TPM1, MYL2 and MYL3. In 135/401 (34%), a MYBPC3 mutation was identified. Of them, 42 were consecutive unrelated probands born in the Mugello valley in Tuscany, who harboured the same MYBPC3 Glu258Lys pathogenic mutation. Haplotype analysis examined in 4 families using 9 intragenic SNPs or STRs, showed a unique haplotype covering a 2Mb genomic region. To confirm these results, haplotype analysis was performed all 42 index cases and 100 healthy adult subjects, born in the same geographical region of Tuscany.
Conclusions: We have identified a novel founder mutation in the MYPC3 gene (Glu258Lys) causing HCM in a Tuscan valley. The presence of a common haplotype suggests that all affected families share the same ancestry.

P12.039
Mitochondrial $tRNA^{Gln}$ (m.4395A>G) mutation manifesting as hypertrophic cardiomyopathy

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Background: Defects of the mitochondrial genome are responsible for a heterogeneous group of phenotypes, including cardiomyopathies (CMPs). Several point mutations of the mitochondrial deoxyribonucleic acid (mtDNA) have been identified as a cause of hereditary CMP. The majority of pathogenic mutations are heteroplasmic, with mutated and wild-type mtDNA coexisting within the same mitochondrion. Homoplasmic mutations are challenging in terms of diagnosis and assigning pathogenicity, as human mtDNA is highly polymorphic.
Hypothesis: The present study concerns a family with hypertrophic cardiomyopathy (hCMP), which segregated with matrilinear transmission.
Methods: Double-stranded DNA products generated for denaturing gel electrophoresis (DGGE) analysis were subjected to direct sequencing using a 48 capillaries Abiprism 3730 sequencer (Applied Biosystems®) with Big Dye terminator v3.1 sequence kit and SeqScape2.5 software for sequence analysis".
Results: The 14-year-old index male presented with hCMP, preexcitation syndrome, and severe ventricular arrhythmias. mtDNA sequencing revealed the m.4395A>G transition in the mitochondrial $tRNA^{Gln}$ gene, which was shown to be homoplasmic by DGGE. The m.4395A>G mutation altered an evolutionary conserved nucleotide and affected a highly conserved U.G pair in the secondary structure of $tRNA^{Gln}$. The mutation was absent in 350 healthy subjects and was

scored as “possible pathogenic” by the Clustal-W-program.
Conclusions: This study shows the association of the homoplasmic m.4395A>G mutation in the $tRNA^{Gln}$ gene with hCMP. To study the impact of this mutation and its contribution to the pathophysiology of mitochondrial hCMP, transgenic mice with distinct mutations could be helpful.

P12.040
Expansion of a diagnostic service for hypertrophic cardiomyopathy using next generation sequencing.

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Since 2003 the Oxford Molecular Genetics Laboratory (OMGL) has provided a diagnostic service for hypertrophic cardiomyopathy (HCM); a condition with an estimated prevalence of 1/500, reported to be the most common cause of sudden cardiac death in young athletes and people under 35 years of age. Currently, mutation screening of the four most commonly associated genes (*MYH7, MYBPC3, TNNT2* and *TNNI3*) is undertaken using a high resolution melt analysis (Lightscanner™)/ Sanger sequencing approach. To date, analysis has been undertaken in >1300 probands; clinical sensitivity in this cohort was ~47%.
A next generation sequencing approach has the potential to increase clinical sensitivity by expanding the number of genes that can be screened in parallel whilst also reducing reporting times for a similar cost.
In collaboration with the OMGL, the Oxford Biomedical Research Centre (BRC) has validated a next generation sequencing protocol. Using the Roche 454 GS-FLX platform a significant increase in clinical sensitivity was observed in a cohort of 231 patients using an expanded gene panel including *TPM1, ACTC1, MYL2, MYL3, CSRP3, PRKAG2, PLN, GLA, LAMP2, FHL1* and the mitochondrial genome in addition to those previously analysed.
Implementation into the diagnostic laboratory requires stringent validation in order to meet quality and regulatory requirements. The process from sample receipt to reporting must be robust to be useful in the routine environment. Here we present the development of a workflow algorithm incorporating robotics and third party analysis software which demonstrates that this sequencing technology has clinical utility as a diagnostic strategy for HCM.

P12.041
Minigene functional assay of a splice mutation of the MYBPC3 gene in hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is a very common genetic cardiac disease and represents the most common cause of sudden cardiac death in the young. The main genes involved are MYH7, MYBPC3, TNNT2, TNNI3, TPM1and ACTC1. HCM is characterized by a marked genetic and phenotypic heterogeneity. In case of novel mutations at splice sites or adjacent regions, analysis of mRNA is virtually unfeasible because of the lack of cardiac tissue, where sarcomeric genes are expressed. Here we report on a minigene assay used to overcome this problem in a case of an undescribed putative splicing mutation in the MYBPC3 gene. A HCM affected newborn, deceased at 15 days, was double heterozygous for two mutations of the MYBPC3 gene c.772G>A and c.2414-1G>A. The paternal mutation c.772G>A was reported as causative of HCM. The c.2414-1G>A mutation, involving a splice site was inherited from the unaffected mother and never reported in the scientific literature. It was absent in 500 control chromosomes. As no cardiac tissue of the proband was available, mRNA could not be tested. Therefore a minigene assay was performed. Fragments corresponding to exons 25-26 of the MYBPC3 gene from the wild type and the mutant alleles were separated by TA cloning using the proband’s mother genomic DNA. The two allelic fragments were inserted into the pSPL3 eukaryotic vector. The plasmids were transfected into HeLa cells. After reverse transcription of the extracted RNA, the resulting cDNAs were sequenced and compared. The mutant construct generated a product in which exon 26 was skipped.

P12.042
Identification of seven novel CRLF1 mutations in Crisponi Syndrome and of a founder mutation in the French gypsy population

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Crisponi syndrome (CS) is a rare and severe autosomal recessive disorder (MIM ID #601378) characterized by contractions of the facial muscles in response to stimuli or during crying, facial dysmorphic features, camptodactyly, major early feeding, respiratory difficulties and hyperthermic episodes, frequently leading to early death from hyperthermia or apnea during the first year of life. CS is caused by mutations in the cytokine receptor-like factor 1 (CRLF1 gene) and is allelic to Cold-Induced Sweating Syndrome type 1 (CISS1). We describe here the clinical and genetic features of ten patients with CS from eight families and seven new CRLF1 mutations. For a further delineation of CS, we performed a meta-analysis of the literature and describe the phenotypic spectrum of all patients with CRLF1 mutations. CS and CISS1 are most probably a single clinical entity with common symptoms and different presentations depending on the patients’ age at diagnosis.
In addition, the homozygous duplication c.713dupC was identified in three patients from French Gypsy families with CS. A common haplotype that segregated with the disease in the three families was detected by haplotype reconstruction with six markers (microsatellites) which span ~4 Mb of DNA covering the CRLF1 locus. In conclusion, we report new CRLF1 mutations and the first founder mutation in the French Gypsy population.

P12.043
The first experience of molecular-genetic analysis of CADASIL in Serbia

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencaphalopathy (CADASIL) is hereditary form of stroke with prevalence of 2/100.000 adults. Beside stroke-like episodes, cognitive disturbance, behavioral abnormalities and migraine with aura are common features of CADASIL. CADASIL is caused by mutations in *NOTCH3* gene and more than 150 *NOTCH3* mutations have been described so far. Majority of them are missense mutations, leading to loss or gain of cysteine and resulting in odd number of cysteine residues within one of the EGF-like domains. It has been shown that almost 90% of mutations occur in exons 2-6. Therefore, as the first step in establishing molecular genetic testing for CADASIL in our country, we performed direct sequencing of those five exons of *NOTCH3* gene. We analyzed DNA samples of 40 patients clinically suspected to CADASIL. DNA sequencing was performed using ABI 310 Genetic Analyzer. Four different mutations in seven patients (one sibling pair, one sibling trio, and two single cases) were detected: Cys65Tyr (exon 2), Gly89Cys (exon 3), Arg90Cys (exon 3) and Ala319Cys (exon 6). According to our data, mutation Cys65Tyr has not yet been described in the literature.
In conclusion, we have successfully started with genetic testing of CADASIL in Serbia. However, relatively low frequency of mutations in tested patients (18.92%) indicates that in the further CADASIL testing stricter clinical criteria should be introduced and also mutation analysis of the remaining *NOTCH3* exons should be developed.

P12.044

Very High Sensitivity Somatic Mutation Detection using Ice COLD-PCR and BLOCker Sequencing

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Transgenomic has developed very high sensitivity methods for detecting somatic mutations notably in the discipline of cancer therapy where key genetic changes are associated with EGFR antagonists' effectiveness, e.g. in the genes EGFR, K-RAS, PIK3CA, BRAF, p53 and NRAS. The novel techniques of Ice COLD-PCR and BLOCker Sequencing preferentially enrich mutant alleles compared to wild-type alleles. These are not allele-specific techniques; therefore all mutations, both known and unknown, are enriched and identified in a single reaction. Together these methods allow mutations at concentrations as low as 0.01-0.05% in background of wild-type to be confirmed by sequencing. Additionally Ice COLD-PCR and BLOCker Sequencing require no special equipment using only standard DNA thermocyclers and Sanger sequencing equipment. Data will be presented showing how these methods allow DNA sequencing confirmation of somatic mutations in samples such as low tumor-load biopsies, formalin-fixed paraffin-embedded slides, fine-needle aspirates, circulating tumor cells and circulating free tumour DNA in plasma and serum. These methods can also be used to confirm low signal Pyrosequencing and next-generation deep-sequencing results. Using these techniques routine, rapid, simple and inexpensive detection of somatic mutations in (1) cancer patients' plasma or serum or; (2) samples where very limited tumour tissue is available, offers a non-invasive option for cancer biomarker detection as well as monitoring remission, relapse and emergence of resistance mutations post-treatment. Finally Ice COLD-PCR and BLOCker Sequencing can be applied to analysis of mixed viral infections and mitochondrial heteroplasmies.

P12.045***

Detection of low frequency variation in heterogeneous samples using an accurate and sensitive next generation sequencing platform

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Cancers often display biological and molecular heterogeneity with a variety of genetic abnormalities. Specific genetic mutations can alter the course of disease progression, metastasis, and drug resistance. Some mutations in cancer are prevalent at a low frequency in clinical samples, often due to contamination of tumor samples with normal alleles from adjacent non-malignant cells. The identification of genetic variants resulting from sub-populations is important to better understand the causal biology of cancer. Second-generation sequencing can identify low frequency alleles resulting from cellular sub-populations. Here we describe the reliable detection of alleles occurring at very low frequencies in heterogeneous samples by utilizing the accuracy of the SOLiD System. We demonstrate that the SOLiD System with Exact Call Chemistry (ECC) provides a highly accurate approach to detecting low frequency variants in cancer research and other applications.

P12.046

Mutation screening of CRYAA, CRYGC, CRYGD and GJA8 genes associated with congenital cataract in Indian patients

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The purpose of this study was to investigate mutations in CRYAA, CRYGC, CRYGD and GJA8 in Indian patients with congenital cataract. Thirty clinically diagnosed congenital cataract cases below 3 years of age from northern India were enrolled in this study. Genomic DNA was extracted from peripheral blood, all coding and exon/intron regions were amplified using PCR and direct sequencing was performed to detect any nucleotide variation. ProtScale and Discovery Studio programs were used for insilico and structural analysis. DNA sequencing analysis of CRYAB, CRYGC, CRYGD and GJA8 showed total of six variations of which two were novel (CRYGC:p.R48H), (GJA8:p.L281C) and four have been previously reported (CRYAB: rs11603779T>G) (GJA8:p.L268L,), (CRYGD:p.R95R, c.T564C). Both the novel changes, in CRYGC and GJA8 genes were found in 16.6% of the patients. Previously reported nucleotide alterations (CRYGD:p.R95R, c.T564C) were found in 90% of the patients. Insilico and structural analysis data suggested that two novel non-synonymous mutations altered the stability and solvent accessibility of CRYGC and GJA8 proteins which may lead to lens opacification. We observed two novel nonsynonymous variations and four reported variations in CRYAB, CRYGC, CRYGD and GJA8 genes. The p.R48H variation in γ-crystallins C may disrupt the normal structure of lens and can cause cataract. Cx50 is responsible for joining the lens cells into a functional syncytium and mutation (p.L281C) in this gene may lead to lens opacification resulting in cataract formation. This study further expands the mutation spectrum of congenital cataract and help understanding how mutant proteins can lead to opacification of lens.

P12.047

Novel missense mutations of RYR2 gene in catecholaminergic polymorphic ventricular tachycardia patients.

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BACKGROUND: Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare inherited heart disease, which can lead to life-threatening ventricular arrhythmias in patients with a structurally normal heart., and may provide a candidate for sudden cardiac deaths CPVT is caused by mutations in the RyR2 gene encoded the ryanodine receptor isoform 2 (RyR2), This calcium release channel is an essential intracellular ion channel that is central to Ca(2+) signaling and contraction in the heart .

METHODS: We screened 35 patients with CPVT and available family members for mutations in the RyR2 gene Mutation screening of RyR2 gene was performed on genomic DNA samples extracted from peripheral blood. PCR amplified fragments covering areas mutative critical exons 2-4, 6-15, 17-20, 39-49, 83, 84, 87-97-105 were analyzed by sequencing. For mapping of the deletions RYR2 exons, we used probes for multiplex ligation-dependent probe amplification (MLPA) analysis.

RESULTS: We identified three novel RyR2 missense mutations M4107V, E4182A and S4122R in three CPVT patients. These RYR2 variants were found in evolutionally conservative area of RYR2 genomic sequence. The carriers of these mutations exhibited frequently syncope, their age of onset was between nine and 10 years and their treatment consists of beta blockers.

CONCLUSIONS: Based on their character it can be assumed that identified sequential changes are causal mutations, thus the mutations responsible for the CPVT phenotype. However, the proposed theory must be proved within a group of 100 healthy controls.

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P12.048

Molecular analysis of the *SEC23B* gene in patients affected by Congenital Dyserythropoietic Anemia Type II (CDAII)

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Congenital Dyserythropoietic Anemia type II (CDAII), the most frequent type of CDA, is an autosomal recessive disease characterized by ineffective erythropoiesis, peripheral hemolysis, erythroblast morphological abnormalities and hypoglycosylation of some red blood cells membrane proteins. Recently, we and others identified mutations in *SEC23B* as responsible for CDAII. SEC23B is a member of the SEC23/SEC24 family, a component of COPII coat protein complex

which is involved in protein trafficking through membrane vesicles from the endoplasmic reticulum to the Golgi apparatus. The aim of the study was to characterize the molecular defect in CDAII patients of Italian origin.
Sixteen CDAII index patients from unrelated families were analyzed by direct exon sequencing. We identified 15 different mutations, four of which were novel (p.Gln214Stop, p.Thr485Ala, p.Val637Gly, p.Ser727Phe). In particular, four patients were carrying homozygous missense mutations and 10 patients were compound heterozygotes (five patients carried a missense and a non-sense mutation and five carried two different missense mutations). The new mutations affected highly conserved aminoacids and were not found in 200 controls. The mutations lead to a 50% decrease in mRNA expression levels of *SEC23B* in lymphocytes and erithroid precursors of patients with missense/non-sense mutations compared to controls. Despite the sequencing of all exons and flanking intronic regions, two patients displayed a single heterozygous mutation, suggesting that mutations could also be located in non-coding regions. Other possibility could be the presence of copy number alterations within the gene. Alternatively, we cannot exclude that a second gene could be involved in the pathogenesis of CDAII.

P12.049
iPS cells to model CDKL5-related disorders

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Rett syndrome is a progressive neurologic disorder that represents one of the most common causes of mental retardation in females. Mutations in *CDKL5* gene have been identified in females with the early onset seizure variant of Rett syndrome and also in males with X-linked epileptic encephalopathy. CDKL5 is a kinase protein highly expressed in neurons, but its exact function inside the cell is unknown. To address this issue we established a human cellular model for *CDKL5*-related disease using the recently developed technology of induced pluripotent stem cells (iPSCs). iPSCs can be expanded indefinitely and differentiated in vitro into many cell types, including neurons. These features make them the ideal tool to study disease mechanisms directly on the primarily affected neuronal cells. We successfully derived iPSCs from fibroblasts of two CDKL5-mutated female patients affected by early onset seizure variant of Rett syndrome and one male with X-linked epileptic encephalopathy. We demonstrated that our female *CDKL5*-mutated iPSCs maintain X-chromosome inactivation and we identified clones expressing either the mutant *CDKL5* allele or the wild type allele. These last clones represent the ideal experimental control since they are genotypically identical to those expressing the mutant allele and differ only for CDKL5 expression. Furthermore, our data indicate that both *CDKL5*-mutated iPSCs can be differentiated into neurons and are thus suitable to model disease pathogenesis in vitro.

P12.050
CFHR5 nephropathy: a new inherited hematuric glomerulopathy with increased frequency in Cyprus due to a founder mutation

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Our team contributed to the molecular investigation of a new autosomal dominant renal disease, seeming to be endemic in Cyprus, which is characterized by microscopic and synpharyngitic macroscopic hematuria, renal failure and C3 glomerulonephritis. The only mutation found thus far in complement factor H related protein 5 (*CFHR5*) gene, is a duplication of exons 2-3 resulting in reduced affinity for glomerular-bound complement.
Here we present clinical data from 16 families, some being very large, with 105 patients sharing this common mutation due to an extended founder effect. Families originate mainly from one of two geographic regions of Cyprus. Heterozygous carriers of the duplication were detected via a simple PCR test using three specific primers. An additional gap PCR test was designed to detect probable homozygous carriers. No homozygous carrier was found. The most striking finding is the big difference between the two genders as regards progression to proteinuria, chronic renal failure (CRF) and end stage renal disease (ESRD). Proteinuria with CRF or ESRD developed on top of hematuria in 68% male patients instead of 14% female patients aged over 50-yo. Fifteen men reached ESRD compared to only three women. The disease penetrance is nearly 90%, as 10 mutation carriers were symptomless.
These data stress the wide clinical spectrum of *CFHR5* nephropathy, a new disease entity, characterized by high risk for renal failure especially in males. Based on our experience, it is probable that some patients with CFHR5 nephropathy and episodes of macroscopic hematuria may be misdiagnosed with IgA nephropathy.

P12.051
A DNA variant within the 3'-UTR of HBEGF alters the regulatory action of hsa-miR-1207-5p and is associated with progression of renal failure in CFHR5 nephropathy

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HBEGF (Heparin Binding Epidermal Growth Factor) is expressed in podocytes and was shown to play a role in glomerular physiology. MicroRNA binding sites on the 3'-UTR of HBEGF were predicted using miRWalk software followed by sequence analysis in 162 patients diagnosed with mild or severe glomerulopathy. A single nucleotide polymorphism, mirSNP C1936T (rs13385) of the 3'-UTR, was identified at the second base of the seed region of microRNA hsa-mir-1207-5p. AB8/13 undifferentiated podocytes were transfected with miRNA mimics of hsa-mir-1207-5p and HBEGF protein levels were reduced by 50%. An extensive sequence containing the mirSNP allele-1936C was then cloned into the pMIR-Report Luciferase vector and co-transfected with miRNA mimics of hsa-mir-1207-5p into AB8/13 undifferentiated podocytes. In agreement with the western blot data, transfection of mimic resulted in reduced luciferase expression demonstrating the ability of hsa-mir-1207-5p to regulate HBEGF expression by direct binding. On the contrary, in the presence mirSNP 1936T allele, this regulation was abolished. Collectively, these results demonstrate that variant 1936T of this mirSNP blocks the ability of hsa-mir-1207-5p to regulate HBEGF expression in podocytes. We hypothesized that this variant may have a functional role as a genetic modifier. Interestingly, in a cohort of 72 patients who were diagnosed with CFHR5 nephropathy, inheritance of the mirSNP 1936T allele was significantly increased in the group who demonstrated faster progression to chronic renal failure (p-value of 0.0485). No similar association was detected in a cohort of patients with Thin Basement Membrane Nephropathy. A larger cohort is required for further validation of these results.

P12.052
Diagnosis of Charcot-Marie-Tooth Disease

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Charcot-Marie- Tooth disease (CMT) is a genetically heterogeneous group of diseases that affect the peripheral nervous system. The most commonly used classification combines clinical findings with the inheritance pattern and either electrophysiological or anatomical pathology findings. The combination of these data allows the identification of several main categories of CMT. The majority of cases can be classified demyelinating forms (or CMT1, characterized by a low NCV in electrophysiological studies), CMT2 (axonal form caused

by specific effects on the axon itself, and conserved NCV) or CMT4 (recessive and severe forms). Recently, disease variants have been described that have intermediate NCV. Each of these types can be further divided into subtypes, depending on the underlying causative molecular defect. However, it should be noted that there is not a good genotype_phenotype correlation and that great variability exists, both within and between families, regarding the degree of clinical expression. The prevalence of CMT ranges from 10 to 30 per 100,000, depending on the geographical region of origin. The most common cause of CMT1 is CMT1A, which results from the duplication of a genomic fragment that encompasses the PMP22 gene on chromosome 17, followed by CMT1B, 1C, 1D and 1E. In the recent years, all the published studies identify CMT2A as the most common cause of CMT2 (which would account for 10- 33% of cases). We propose a diagnostic CMT algorithm including the recent molecular findings that allows the diagnosis of more than 80% of CMT1 patients and more than 40% of CMT patients.

P12.053
Duplication of cis-regulatory DNA elements downstream of SHOX gene causes Leri Weill Syndrome

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Leri-Weill syndrome (LWS) is a dominantly inherited skeletal dysplasia ascribed to haploinsufficiency of the SHOX protein caused by deletions or point mutations of the gene and to heterozygous deletions downstream the intact SHOX gene, involving conserved non coding cis-regulatory DNA elements (CNEs) showing enhancer activity.
We describe the first cases of "pure" duplications of the CNEs-containing region downstream the SHOX gene in two LWS families, suggesting the duplication of the cis-regulatory DNA elements as the possible cause of the LWS phenotype.
The precise mechanisms of the pathogenic effect caused by the microduplication of the CNEs-containing region are unclear. The disturbance of the long range regulation of gene expression, essential for producing the gene product in the proper time and place and in the correct quantity, has been proposed as the cause of a number of diseases in which the suspected target genes appeared to be completely normal. It is possible that duplication exerts a negative effect on SHOX transcription. Recently, it has been demonstrated that the proper gene regulation depends not only on the required transcription factors and associated complexes being present but also on the integrity, chromatin conformation and nuclear positioning of the gene chromosomal segments. On the basis of the above considerations it is not unlikely that the duplication alters the three dimensional chromatin spatial organization impeding in some way the enhancer to contact the promoter, resulting in a lack of gene activation resulting in a condition of haploinsufficiency.

P12.054
Screening for *COL4A3/COL4A4* mutations in 122 familial and sporadic cases of microscopic hematuria

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Recently described mutations in glomerular basement membrane (GBM) related genes (*COL4A3, COL4A4, LAMB2*) in patients with hematuria and severe nephrotic syndrome give more evidence for the significance of the GBM in glomerular filtration. Familial microscopic hematuria, thought to be a benign disease, seems to be a frequent condition and *COL4A3/COL4A4* mutations explain about half of the cases. However, recent data from our laboratory subsequently confirmed by others, show that more than 50% of these patients develop chronic renal failure and even end-stage renal disease. We believe that this phenotypic heterogeneity is due to different mutation types or due to modifier genes action.
In order to investigate the "different mutation type" hypothesis and find the frequency of *COL4A3/COL4A4* inherited and *de novo* mutations we set up a *COL4A3/COL4A4* mutation screening in a large cohort of 122 familial and sporadic hematuria cases. The exons and flanking intronic regions of *COL4A3/COL4A4* are amplified with appropriate primers. Using SURVEYOR endonuclease for mutation detection we will have the genetics results in order to be able to investigate deeper the functional role of the collagen IV mutations. SURVEYOR digestion cleaves at mismatched base pairs in heteroduplexes. If cleavage is evident (on agarose electrophoresis), DNA re-sequencing is performed.
Preliminary results of our screening are the polymorphisms *COL4A3*-G43R, *COL4A3* IVS2+12GT, *COL4A3*-P74P, *COL4A3*-P414L and *COL4A3*-E162G. Possible phenotypic contribution of these SNPs as a functional haplotype cannot be excluded. Appropriate cell culture studies will be designed to investigate the functional role of polymorphisms and mutations in *COL4A3*, and *COL4A4* chains.

P12.055
A novel NDUFV1 gene mutation associated with complex I deficiency by a genome-wide approach in consanguineous siblings with brainstem lesions and Leigh syndrome

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While deficiency of complex I of the mitochondrial respiratory chain is a frequent cause of encephalopathy in children, only a few mutations have been reported in each of its subunits. Furthermore, in the absence of families large enough for conclusive segregation analysis, and of robust functional testing, it is difficult to unequivocally demonstrate the causality of the observed mutations and to delineate genotype-phenotype correlations, making additional observations of these very rare genetic defects necessary. We observed two siblings with an early onset encephalopathy with medulla, brainstem and mesencephalon lesions on brain MRI and death before eight months of age, caused by a complex I deficiency. The parents were first cousins and unaffected. Assuming that the disease was caused by homozygosity for an ancestral mutation in a nuclear-encoded gene, we used homozygosity mapping and identified 4 genomic regions that were homozygous and concordant in the two patients, encompassing 4 complex I genes, one of which only, NDUFV1, showing a missense mutation. The mutation, p.Arg386His, affects a highly conserved residue, contiguous to a cysteine residue known to coordinate a Fe ion. Another mutation of the same codon had been reported in similarly affected patient, yielding additional strong evidence for causality. This observation adds to our understanding of complex I deficiency. It validates the important role of Arg386, and therefore supports the current molecular model of iron-sulfur clusters in NDUFV1.

P12.056
Prevalence of Congenital Adrenal hyperplasia (CAH), due to 21-hydroxylase deficiency, in a Jordanian sample pool

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CAH represents a family of autosomal recessive disorders. About 95% of the CAH cases are caused by 21-hydroxylase deficiency, resulting from a defect in the *CYP21A2* gene. The main aim of this study was to screen the Jordanian population for the 8 most common small *CYP21A2* mutations and introduce a prenatal and a neonatal genetic test for CAH in Jordan. Current sample study consisted of 37 clinically diagnosed index patients, including 20 males and 17 females. In most cases, DNA samples for family members were available, bringing the total number of samples to 140. As a screening method we implemented the amplification refractory mutation system (ARMS), identifying mutations in 33 index patients, 15 of which were heterozygotes for a single mutation, 5 were compound heterozygotes, 11 were homozygotes and 2 were compound homozygotes. Mutation

frequency was the highest for In2cs mutation at 37.8%, followed by Q318X at 24.3% and 13.5% for 8bp deletion. Also, in 25% of CAH cases due to *CYP21A2* gene defect, mutations are caused by large gene deletions (i.e. 30 kb gene deletion). Hence, to be able to obtain a true statistical representation of *CYP21A2* mutations in the Jordanian population, our sample pool needs to be tested for the 30 kb deletion. This will be achieved through Multiple Ligation-dependant Probe Amplification (MLPA) method, utilizing a CAH-MLPA kit. Once the analysis is complete, we can implement a prenatal and a neonatal testing on a national scale, hoping to reduce the overall incidence of CAH in a Jordanian population.

P12.057
Identification of CYP21A2 mutations in Czech patients with 21-hydroxylase deficiency - structural analysis of the chimeric CYP21A1P/CYP21A2 gene

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Congenital adrenal hyperplasia (CAH) comprises a group of autosomal recessive disorders caused by an enzymatic deficiency. Approximately 90% of all CAH cases is associated with mutations in the steroid 21-hydroxylase gene (*CYP21A2*). The *CYP21A2* gene and its inactive pseudogene (*CYP21A1P*) are located within the HLA region on chromosome 6p21.3. Their intergenic recombinations are responsible for about 95% of mutations.

In 267 Czech probands with 21-hydroxylase deficiency were identified 30 different *CYP21A2* mutant alleles (4 of them were not described so far). The most frequent mutation, a chimeric *CYP21A1P/CYP21A2* gene, was found in 33,7% of mutant alleles (a new type designated CH-7 was characterized). Small DNA rearrangements of the *CYP21A2* gene were present in 59,2% of mutant alleles (3 novel point mutations were detected). Total deletions of *CYP21A2* were detected in 4,9% and duplications of *CYP21A2* associated with a mutation on both copies were detected in 0,4% of mutated alleles.

In the set of 90 patients, we identified four types of chimeric *CYP21A1P/CYP21A2* genes. The most common type was the newly characterized CH-7 type (21,4% of mutant alleles). We performed a detailed sequence analysis of chimeric *CYP21A1P/CYP21A2* genes to determine the breakpoints in *CYP21A1P-CYP21A2* conversion areas. All chimeric genes have the CYP21A1P promotor and p.Pro30Leu mutation in exon 1 but differ in the presence of other mutations and polymorphisms.

Mutations in *CYP21A2* gene were determined using a long-range PCR, secondary PCR and restriction analysis, direct sequencing, and MLPA method.

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P12.058
Molecular Diagnosis of Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency

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Introduction: 21-hydroxylase deficiency is the most common cause of congenital adrenal hyperplasia (90%-95% of cases), a family of autosomal recessive disorders with highly variable phenotypical expression involving impaired synthesis of cortisol from cholesterol by the adrenal cortex. The CYP21A2 gene is located on 6p, within the HLA complex, a region that displays a complex organization of genes with great variability in gene size and copy numbers. Approximately 30 kb from the functional gene of 21-OHD is located a nonfunctional pseudogene (CYP21A2P) that shares significant sequence homology with CYP21A2. Due to the complexity of large rearrangements that can affect *CYP21A2* and its pseudogene, correct genotyping of *CYP21A2* gene is difficult and requires not only the identification of *CYP21A2* common mutations but also the detection of *CYP21A2* deletions, *CYP21A2P/CYP21A2* chimeric genes (20% of mutant alleles, resulting from the common 30-kb deletion) or *CYP21A2* duplications (7% of the alleles). Therefore we developed a testing approach that combines the detection of *CYP21A2* common point mutations and large rearrangements, and allows the detection of approximately 80-98% of *CYP21A2* mutations associated with CAH.

Method/Results: 22 samples were tested by locus-specific PCR/multiplex minisequencing and MLPA (P050, MRC-Holland): 4 had no mutations, 9 had point mutations, 2 had large deletions and 7 had a common point mutation combined with a large deletion or duplication.

Conclusion: The present work stresses the importance of considering detection of *CYP21A2* point mutations and large rearrangements (particularly *CYP21A2* duplications that can be responsible for some false positive cases), for CAH diagnosis and counselling.

P12.059
First case report of *PHOX2B*-confirmed congenital central hypoventilation syndrome in Russia

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Congenital central hypoventilation syndrome (CCHS) is a rare autosomal dominant disorder of the autonomic nervous system characterized by the absence of adequate control of respiration with decreased sensitivity to hypoxia and hypercapnia. The paired-like homeobox 2B (PHOX2B) gene has been identified as the major gene involved in CCHS. Case presented in the newborn period with multiple episodes of apnoea and respiratory insufficiency that required ventilatory support. There was no family history of CCHS. A heterozygous insertion of 21 bp DNAs for coding 7 alanines was detected in exon 3. Analyses of the parents' *PHOX2B* genes were normal. To the best of our knowledge, this is the first report of clinical description and confirmation of CCHS by molecular genetic testing in Russia.

P12.060
Novel and recurrent *JAG1* mutations in patients with tetralogy of Fallot

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Mutations in the ligand of Notch signaling pathway Jagged1 (*JAG1*) have been identified in patients with Alagille syndrome (AS) and in some patients affected by apparently non-syndromic tetralogy of Fallot (TOF). We analyzed the entire coding sequence of *JAG1* gene to determine the occurrence of small deletions, insertions and point mutations in 123 TOF individuals. None of the patients presented clinical features of AS, all cases had normal standard karyotype and 22q11 deletion was excluded. The patients' cohort included 112 subjects with non-syndromic TOF and 11 with multiple anomalies in the setting of an unidentifiable disorder. Mutation analysis of *JAG1* gene using dHPLC identified three different, possibly pathogenetic, aminoacidic substitutions in 4 unrelated patients, including 3 sporadic cases and one with familial congenital heart disease. One of the *JAG1* sequence changes (p.Gly309Arg) was novel, while two (p.Pro810Leu, p.Arg937Gln) were known. The p.Pro810Leu mutation was identified in a patient with TOF, cleft lip and palate and mild mental impairment, while p.Gly309Arg and p.Arg937Gln variants were detected in 3 patients affected by isolated TOF (3/112, 2.7%). All identified variants were excluded in 300 controls. The present results expand the *JAG1* mutational and phenotypic spectrum and confirm that these mutations occur in a subset of non-syndromic TOF individuals.

P12.061
High frequency of *NOTCH1* mutations in familal LVOTO : a study of 324 probands and their families

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Background: Left ventricular outflow tract obstructions (LVOTO) are highly heritable congenital heart diseases, with bicuspid aortic valve

(BAV), aortic valve stenosis (AVS), coarctation of the aortae (COA) and hypoplastic left heart syndrome (HLHS) being the most frequent diagnoses. The BAV may be undetected, but has a risk for serious complications and sudden cardiac death. Mutations in *NOTCH1* are reported to cause LVOTO, but data on prevalence and phenotypic spectrum in a large cohort have not been published.

Methods/Results: *NOTCH1* mutation screening was performed in a well documented cohort of 324 probands with LVOTO. Sixteen mutations were detected (5%): eight mutations are considered disease causing (one deletion, five truncating and two splice site mutations). Two of these 8 mutations are de novo, the other six occur in familial disease (6/76= 8%). Moreover we found 8 possible causative non-synonymous mutations. Apart from these 16 mutations, 69 unclassified variants were detected. The relatives of all mutation carriers were screened for mutations and for cardiac defects. We detected besides LVOTO, also non-penetrance, tetralogy of Fallot and pulmonary valve stenosis/atresia

Conclusion: Firstly, disease causing *NOTCH1* mutations are detected in 8% of familial LVOTO. Secondly, *NOTCH1* mutations cause all subtypes of LVOTO, but non-penetrance or right sided defects may occur like tetralogy of Fallot and pulmonary valve atresia. We recommend *NOTCH1* mutation screening in patients with LVOTO, especially in familial LVOTO, because it will help to detect relatives at risk for complications of a previously unknown heart defect and at risk for affected offspring.

P12.062
Centronuclear myopathy: 38 novel DNM2 mutations reveal hotspots and a genotype/phenotype correlation

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Centronuclear myopathies (CNM) are associated with general skeletal muscle weakness. The histopathological findings involve muscle fiber atrophy, type I fiber predominance and abnormally centralized nuclei. The severe X-linked and the milder autosomal recessive CNM result from mutations in MTM1 and BIN1, respectively. The autosomal dominant form with mild to moderate etiopathology and childhood or adult onset is linked to DNM2 mutations. Specific DNM2 mutations are also associated with a peripheral neuropathy, Charcot-Marie-Tooth (CMT) disease, the most common inherited neurological disorder. DNM2 (19q13) encodes dynamin 2, a mechanochemical enzyme hydrolyzing GTP to apply forces on membranes and is therefore a key player for membrane trafficking at the plasma membrane and the trans-Golgi network.

Fourteen different CNM-related DNM2 mutations in 39 families have been reported so far. In this study we describe a cohort of 38 novel autosomal dominant CNM families harbouring 8 known and 2 new mutations including the first splice mutation, thereby expanding the genotypic CNM spectrum and confining regions more prone to mutations. Combining our results with the published data, distinct mutation hotspots became apparent in exons 8, 11, 14 and 16 and allow a reliable genotype-phenotype correlation. Notably, exon 16 mutations are invariably associated with a severe neonatal phenotype. None of the mutations are associated with both, CNM and CMT, suggesting that the pathomechanisms are amino acid-dependent and not linked to modifier genes. We established a sequencing hierarchy for hotspot-containing exons based on the patients clinical data and thereby help to guide genetic counselling and molecular diagnostics.

P12.063
The molecular genetic study of gene *CFTR* for CF patients from Chuvash Republic

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Cystic fibrosis (CF) is a common and generally severe autosomal recessive disorder of the European population. It's caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR) on chromosome 7q31.

In this work we analyzed 11 CF Chuvashes families, consisting of 12 patients with classic form of CF and their 25 relatives.

We'd designed the multiplex system of direct PCR analysis and the multiplex system based on allele-specific ligation reaction PCR analysis and used it for most frequent mutation screening (ΔF508, ΔI507, CFTRdele2,3, 1677delTA, 2143delT, 2184insA, 394delTT, 3821DelT, L138ins, G542X, W1282X, N1303K, R334W, 3849+10kbc>t, 604insA, 3944 delTG, S1196X, 621+1g>t). There were found two CFTR mutations ΔF508 for two CF patients in this work.

We analyzed all patients and relatives by four intragenic SNP markers IVS1CA, IVS6aGATT, IVS8CA and IVS17BCA. The haplotype 22-7-16-13 was observed for 6 patients in the homozygous state and for 4 patients in the heterozygous state. By sequencing of the CFTR gene coding regions and exon-intron junctions the p.E92K mutation was found in homozygous state for two CF patients. The p.E92K mutation was determined for 10 patients with haplotype 22-7-16-13: 6 patients in the homozygous state, 2 patients in heterozygous state in compound with mutation ΔF508 and 2 patients in compound heterozygous with unidentified mutation.

After analyzing 343 unaffected unrelated Chuvashes we identified 5 heterozygous state p.E92K mutation and 4 heterozygous state ΔF508 ones.

P12.064
Multicenter validation study of a novel StripAssay for cystic fibrosis

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Cystic fibrosis (CF), with an incidence of approximately 1 in 3000 live births in Caucasians, is caused by mutations in the cystic-fibrosis transmembrane conductance regulator (CFTR) gene. To date, more than 1500 CFTR mutations have been described, the majority being very rare or private. Worldwide, the most frequent mutation F508del accounts for 30-72% of CF chromosomes depending upon ethnicity. Overall there is great heterogeneity in the remaining pathogenic mutations, as type and distribution vary substantially between populations.

We have developed a reverse-hybridization assay for the rapid and simultaneous analysis of 23 CFTR mutations recommended by the ACMG plus 10 additional ones prevalent in different parts of Europe, as well as the IVS8 polyT (5T/7T/9T) variants. The CF StripAssay, showing a coverage of 70-93% almost all over Europe, is rapid, simple and accessible to automation. The test requires very small amounts of samples, which is of particular importance for prenatal diagnosis and newborn screening. In the first phase of a multicenter validation study genotyping results from 96 out of 111 (86.5%) previously tested samples were confirmed by the CF StripAssay. The remaining discrepant genotypes were due to missing mutant probes on the teststrips. Currently the assay is validated prospectively under routine diagnostic settings in the participating centers. (oberkanins@viennalab.co.at)

P12.065
IFRD1: a possible modifier gene for Cystic Fibrosis Lung Disease

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Cystic fibrosis (*CF*), the most frequent autosomal recessive disease in the Caucasian population, is a lethal multi-system disorder caused by mutations in the CFTR gene. Severity of clinical manifestation, particularly, the pulmonary phenotype, is widely variable, even among patients with the same CFTR genotype or within the same family. This variability is only partially explained by allelic heterogeneity at the CFTR gene, therefore many genes have been investigated as possible CF phenotype modifier. Literature data reports the IFRD1 (*Interferon-Related Developmental Regulator 1*) gene as a possible modifier of cystic fibrosis lung disease severity (Nature 458:1039-42, 2009).
In this study we analysed the three SNPs (rs7817, rs3807213, rs6968084) of IFRD1 gene, previously described in association with a more severe lung phenotype, to confirm these data in a North-East Italian population.
To this purpose,146 CF patients attending the Veneto Regional CF Center of Verona were enrolled. All patients were diagnosed according to international criteria and clinically evaluated for respiratory and gastrointestinal parameters.
SNPs were genotyped by PCR and restriction enzyme analysis.
An association study between IFRD1-polymorphisms and qualitative parameters relatively to respiratory and gastrointestinal traits in CF patients was done. A significant association between the rs7817-CT heterozygote genotype and a higher FEV_1 value was found (p=0.0307). No other significant association was observed.
These results confirm that genetic variations in the IFRD1 gene, or at least other mutations in linkage disequilibrium with the studied SNPs, may contribute to the modification of pulmonary phenotype severity in CF patients.

P12.066
Next generation sequencing with Ion Torrent's PGM™ sequencer and the 5500 SOLiD™ system: targeted resequencing of the *CFTR* gene using sample multiplexing

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Using high-throughput next generation sequencing platforms for resequencing of the *CFTR* gene requires highly accurate data in the coding and flanking intron regions of the gene. While PCR has proven to be a reliable method for amplification of gene specific target regions, it suffers from limited multiplexing capability. We describe a pilot study comprising 20 human samples with different known *CFTR* gene mutations, using a Multiplicon™ multiplexing assay for the *CFTR* gene, sample barcoding and subsequent Next-Generation Sequencing with the 5500 SOLiD™ System and the semiconductor-based IonTorrent Personal Genome Machine™ Sequencer. Results of the samples obtained from the two next generation sequencing platforms were validated by Sanger sequencing.

P12.067
Case of a simultaneous carriage of mutations R408W of *PAH* gene and delF508 of *CFTR* gene.

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In Western-Siberian region cystic fibrosis (CF, MIM 219700) and phenylketonuria (PKU, MIM 261600) diseases meet on the average frequency 1:4000-1:7000 and 1:6000-1:10000 newborns accordingly. Molecular-genetic inspection of patients CF and PKU, living in the Novosibirsk region, has shown that frequency delF508 of *CFTR* gene among all mutations of this gene makes 0,425, and frequency R408W of *PAH* gene - 0,638. These mutations are revealed at patients, both in the homozygous form, and in a compound with other rare mutations.
At inspection of 246 healthy inhabitants of the Novosibirsk region the genital age, selected by epidemiological criteria, the R408W of *PAH* gene and delF508 of *CFTR* gene among all alleles of these genes have made frequencies of 0,0061 and 0,0041 accordingly. Testing mutations R408W of *PAH* gene and delF508 of *CFTR* gene has high diagnostic value at inspection of families burdened on CF and PKU and for revealing of heterozygotic carriers of the given diseases during mediko-genetic consultation.
In our practice in a family surveyed concerning reproductive health, at the woman presence in the heterozygotic form simultaneously mutation R408W of *PAH* gene (inherited from the father) and mutation delF508 of *CFTR* gene (inherited from mother) has been established. Thus, the patient is the carrier simultaneously two hereditary diseases (CF and PKU), and her family needs genetic inspection at planning and pregnancy conducting.The given fact confirms the importance testing mutations of the *PAH* and *CFTR* genes at mediko-genetic consultation of families planning pregnancy.

P12.068
Identification of *CFTR* splicing defects from mRNA analysis

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Cystic fibrosis (CF; OMIM 219700) is a multi-systemic disorder produced by mutations in the *CFTR* gene (*ABCC7*; OMIM 602421). So far, over 1,600 mutations determine its variable phenotypic expression. Splicing mutations account for 15% of CF alleles. However, missense, nonsense and synonymous SNPs have been found to affect the efficiency of the splicing process in CF and other genetic disorders. DNA analysis has allowed us to characterize 97% of the CF alleles; however, the effect of most mutations is not well understood. *In silico* analysis has being applied to different kind of mutations, although the information provided by the different software, sometimes controversial, needs further studies to be corroborated. The aim of this work is to define the optimal conditions to the implementation of mRNA analysis in our current CF strategy to assess the effect of mutations already identified as well as to search undetected mutations in our population. To this aim, nasal epithelial cells were collected by brushing from CF patients and controls. Total mRNA was extracted and reverse transcription performed following standard protocols. Six PCRs overlapping the complete *CFTR* mRNA (6.1kb) have been developed to identify normal and aberrant transcripts in 30 samples. The PCR products were sequenced to corroborate the nature of transcripts. Our results suggest that mRNA analysis is a powerful molecular tool to detect alterations in the splicing process and could contribute to the development of new therapeutic strategies in Mendelian disorders.
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P12.069
Induced pluripotent stem cells (iPSC) from a lissencephaly patient with a DCX gene mutation

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Congenital neurological and neurodevelopmental disorders constitute a heterogeneous group affecting approximately 3% of the population. Genetic factors play a major role in a large proportion of cases. Studies of the underlying disease mechanisms have been hampered by the unavailability of the appropriate tissues as well as the lack of model systems. We have established somatic cells from a patient with a *DCX* gene mutation resulting in lissencephaly, a neuro-developmental disorder characterized by a smooth cerebral surface (pachy- or agyri), mental retardation and seizures. The disease is caused by insufficient migration of maturing neurons. Patient-derived fibroblasts were reprogrammed through forced expression of four transcription factors (Oct4, Kfl4, c-MYC and Sox2) and the resulting iPS cells (Liss-iPSC) were characterized by RT/PCR, immunostaining, karyotyping, methylation analysis as well as embryoid body and teratoma formation assay. Patient derived Liss-iPSC express stem cell markers and show de-methylation of the NANOG and OCT4 promoters and a normal karyotype. Analysis of a teratoma obtained after injection of Liss-iPSC

into a SCID-mouse showed all three germ layers. Liss-iPSC will be differentiated into neuronal cells and neuronal subtypes will be scored for growth, morphology, migration and apoptosis. iPS cells from normal individuals are used as controls. The expected results will provide novel and basic understanding about pathophysiological mechanisms underlying lissencephaly.

P12.070
Molecular analysis of the FOXP2 gene in Italian patients affected by specific language impairment

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The specific language impairment (SLI) are defined as primary disorders of language development. They are not associated with significant difficulties in cognitive development and relationship, neuromotor or neurosensory disorders and social deprivation. They are interested in children over the age of 4 years when the expression language is relatively developed. Because FOXP2 gene on 7q31 has been implicated in the etiology of severe speech and language disorders, we searched for FOXP2 gene mutations in 61 children with clinical diagnosis of SLI.

All probands underwent metabolic, neuro-physiological, psychometric and psycho-linguistic studies, as well as molecular analysis of the FOXP2 gene for the detection of point mutations. We also studied 5 intragenic microsatellites (7-113847; 7-113941; 7-114008; 7-114072; 7-114115) for the detection of maternal uniparental disomy. To search for deletions or chromosomal translocations involving the region of chromosome 7q containing the FOXP2 gene, we analyzed the DNA samples of the patients by FISH. The data were compared with normal control samples.

The analysis of the FOXP2 gene revealed no mutations in all patients included in our study. In 5 patients, we found two polymorphisms (IVS3+33 T>A; IVS5 +17 T>G) previously described. In 4 subjects, a nucleotide substitution (A183T) was detected. Moreover, by FISH and uniparental disomy analysis, we found no chromosomal aberrations.

Our data underline that the role of FOXP2 gene could be no essential in severe speech and language disorders and that could be necessary to examine other genes.

P12.071
Spectrums and frequencies of GJB2, GJB6, GJB3, 12SrRNA, tRNASer(UCN), SLC26A4, SLC26A5 and MYO7A genes mutation among patients with non-syndromic hearing loss from Bashkortostan (Russia)

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Introduction: Congenital hearing loss/deafness is the most common defect and the most prevalent sensorineural disorder in different countries but currently only a minority of genes is included in genetic diagnostics. Mutations in *GJB2*, *GJB6, GJB3, 12SrRNA, tRNASer(UCN)*, *SLC26A4* genes are responsible for more than half of all cases of prelingual nonsyndromic recessive deafness in Caucasians.

Method: Using a custom microarray panel (Arrays HHL - Asper Biothech) that contains 198 point mutations, identified in 8 main genes involved on congenital deafness it is possible to identify the molecular basis of the most common forms, both syndromic and nonsyndromic. The DNA samples of 204 unrelated patients' non-syndromic hearing loss from Bashkortostan had been analyzed.

Result: In group of 204 tested patients 97 patients (47,5%) were either homozygous of compound heterozygous, 56 patients (27,5%) carried only one detected mutation. Allelic frequencies for mutations were: *GJB2* (c.35delG (34%), c.35dupG (0,5%), c.167delT (2%), c.235delC (0,74%), q.-3179G>A (1,23%), c.358_360delGAG (0,5%), c.302A>G (0,25%), c.290_291insA (0,25%), c.299_300delAT (1%), c.333_334delAA (0,5%), c.310_325del14 (0,5%), c.312_327del14 (3%), c.101T>C (1%), c.109G>A (0,5%), c.139G>T (0,25%), c.380G>A (0,25%), c.224G>A (0,5%), c.95G>A (0,25%), c.551G>C (0,5%), c.250G>C (0,25%), c.79G>A+c.341A>G (0,25%)), *12SrRNA* (m.961insC$_{(n)}$ (0,25%), m.961delTinsC$_{(n)}$ (0,74%)), *tRNA*$^{Ser(UCN)}$ (m.7444G>A (0, 25%)), *SLC26A5* (q.-53-2A>G) (0,25%), *SLC26A4* (q.919-2A>G) (0,25%). Discussion: *GJB6, GJB3, 12SrRNA, tRNASer(UCN)*, *SLC26A4, SLC26A5* and *MYO7A* genes mutations are not major causes of hearing loss in patients with non-syndromic hearing loss from Bashkortostan. We suggest other genes may be causes of non-syndromic hearing loss in our region. However, *GJB2* gene should be checked for all patients, at first.

P12.072***
Brown-Vialetto-Van Laere syndrome: broadening the phenotypic spectrum

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We have recently discovered that mutations in *C20orf54* are the principal cause of Brown-Vialetto-Van Laere syndrome (BVVLS), an autosomal recessive disorder characterised by progressive ponto-bulbar palsy and sensorineural deafness. The hearing loss is of insidious onset and precedes the neurological signs. The neurological phenotype is complex and precipitated by the onset of cranial nerve palsies in a previously healthy individual, followed by anterior horn neuronopathy and long tract signs. The disease course is progressive, but the rate of decline is variable. Rapid development of diaphragmatic weakness is a frequent complication in children and cor pulmonale secondary to chronic hypoventilation in adults.

There is increasing evidence that Fazio-Londe disease (FLD), clinically similar to BVVLS except for the deafness, is also caused by mutations in *C20orf54* and that BVVLS and FLD are effectively the same entity. We explored the possibility that some cases of Spinal Muscular Atrophy with respiratory distress (SMARD) may also be caused by mutations in this gene. SMARD patients present at birth with distal muscle weakness followed by sudden onset of respiratory distress secondary to diaphragmatic weakness. About 10% of SMARD patients carry mutations in *IGHMBP2*. We tested 95 *IGHMBP2* negative patients and confirmed that 5% harboured mutations in *C20orf54*.

We give an overview of the clinical features of BVVLS, FLD and SMARD, provide some evidence of genotype/phenotype correlation and discuss the clinical overlap. In view of the management implications, we emphasise the importance of considering mutations in *C20orf54* as the cause of motor neuronopathy in infancy and childhood.

P12.073
Detection of deletions/duplications in alpha-thalassemia by MLPA

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Although Iran is one of the countries with high frequency of thalassemia, few studies have been carried out on detection of different mutations in alpha thalassemia. There are patients suspected of having alpha thalassemia based on hematological indices but no abnormality in globin has been found by gap-PCR for the most common α-thalassemia deletions and no point mutations detected by ARMS and sequencing of the alpha genes . More studies are required to determine the mutation type in these patients. So the aim of the present study was to determine the mutations at the alpha globin gene cluster using MLPA (Multiplex Ligation-dependent Probe Amplification) method in patients who were suspected to be alpha thalassemia carrier but no mutations had been detected in them by conventional techniques.

Twenty patients, whose mutations were not identified, were selected. In addition, 10 samples in which deletions had been confirmed previously by gap-PCR and 5 normal samples were chosen as positive and negative controls respectively and. Following confirmation of the positive and negative controls by MLPA, suspected samples were investigated.

MLPA results demonstrated mutations in 15% of the cases which had been remained undetected by the conventional methods. One case showed the deletion of regulatory element HS-40 and in two other cases alpha triplication in alpha genes were determined.

In conclusion ,the simplicity, high accuracy ,low cost of MLPA makes

this method a superior alternative to gap-PCR for detecting common known and unknown deletions and duplications in alpha-thalassemia.

P12.074
The fifth DFNX1 family - possible neurodevelopmental disturbances and minor visual symptoms associated with reduced PRS enzyme activity

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Only 1-5% of nonsyndromic hearing impairment is X-linked. To date, four loci, DFNX1-4, have been mapped to the X-chromosome. The gene for DFNX1, *PRPS1*, was identified in 2010. The clinical phenotype in DFNX1 is related to an inborn error of purine metabolism in which the enzyme activity of phosphoribosylpyrophosphate synthetase (PRS) is reduced. Mutations in *PRPS1* have also been identified in Arts syndrome, X-linked Charcot-Marie-Tooth type 5 (CMTX5) and PRS superactivity. In Arts syndrome, the enzyme levels are close to zero affecting hearing, vision and psychomotor development. In CMTX5, the level is reduced resulting in peripheral neuropathy, hearing and visual impairment. In PRS superactivity, the high enzyme activity can be associated with hearing loss, hypotonia, ataxia, urinary stones and gouty arthritis.

Today, only four DFNX1 families have been published. The hearing impairment found in the studied male family members was highly variable ranging from congenital profound to progressive postlingual hearing impairment.

We present an five-generation Swedish family with postlingual, progressive X-linked hearing loss associated with a novel c.35A>T (p.H12L) mutation in *PRPS1*. Both affected male family members and female carriers had reduced PRS enzyme activity whereas unaffected family members had normal enzyme activity. No clear signs of auditory neuropathy or vestibular dysfunction could be found. The clinical picture in this fifth DFNX1 family also included strabismus leading in untreated cases to unilateral amblyopathy. In one individual, reduced gross and fine motor skills were noted. These additional findings could be related to the reduced PRS enzyme activity.

P12.075
Spectrum of point mutations in Czech DMD/BMD patients and their phenotypic outcome

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Duchenne and milder Becker muscular dystrophies (DMD/BMD) are X-linked recessive neuromuscular diseases. Both are caused by mutations in the DMD gene. DMD is typically connected with mutations causing premature stop codon creation, while BMD is typically connected with in-frame deletions or duplications. Excluding 60% of deletions and 5% of duplications detected in DMD gene, we identified 63 different point mutations in 69 DMD/BMD patients and DMD/BMD female carriers. For mutation screening on the RNA level, we used reverse transcription-PCR, protein truncation test, and DNA sequencing. For screening on DNA level, we used PCR and sequencing. We describe patients with a mutation creating a premature termination codon but with a mild BMD phenotype, which present three different ways of rescuing the DMD phenotype. In one patient we detected the insertion of a repetitive sequence AluYa5 in intron 56, which led to skipping of exon 57. On the other hand, we present two patients with mutations deep inside the intron leading to severe DMD phenotype by creating pseudoexon and leading to frame-shift. Among presented mutations, there are 32, which are typical for Czech population.

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P12.076
Dentatorubral pallidoluysian atrophy: clinical and genetic analysis of a Sicilian pedigree

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We describe clinical, radiological and genetic features of a three generation Sicilian family affected by DRPLA. We obtained clinical and genealogical information from twelve people of a three generation pedigree. Five out of seven affected living people were examined and blood samples were obtained. Neurological evaluations, neurophysiological and neuropsychological examinations, neuroimaging studies were performed. Genomic DNA was extracted from peripheral blood by standard methods. Triplet expansion in DRPLA was detected by PCR amplification with fluorescent primers. PCR products were separated onto a capillary sequencer (3130XL Applied Biosystems). The proband of the family, a 48-year-old man, at the age of 37, began to experience episodes of loss of consciousness with generalized seizures. Afterwards he developed an unsteady gait, involuntary movements of the head, face and limbs, in addition to cognitive and psychiatric symptoms. Neurological examination was characterize by choreiform movement, myoclonus and cognitive deterioration. His gate was ataxic and tandem walking was impossible. Cranial MRI showed mild diffuse atrophy. Somatosensory,motor and brainstem auditory evoked potentials were altered. Two sisters of the proband reported similar symptoms with different degree of gait, speech disturbance and choreoathetosis. The first son of the proband, a nephew and two young first cousins, at the age of 15 started to complain of myoclonic epilepsy, and variable degree of cognitive disturbance. Molecular analysis of all affected people revealed a heterozygous CAG repeats expansion in ATN-1 gene. We provided a detailed description of the main clinical, genetic and radiological features of the second Italian family with DRPLA.

P12.077
Detection of deletions and duplications in Dystrophin gene of male patients by Multiplex Ligation- dependent Probe Amplification (MLPA) method

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Duchenne muscular dystrophy and Becker muscular dystrophy are X-linked recessive disorders caused by mutations in dystrophin gene. DMD is more severe disease with progressive deterioration in muscle power, and most patients become confined to a wheelchair by the age of 12 and die early in their third decade of life from respiratory or cardiac failure.

BMD is a milder phenotype with age of onset around 12 years.

In the majority of DMD/BMD patients, the disease is due to deletions and duplications in the dystrophin gene. Determining the exact size of deletions and duplications can help to predict frameshift and phenotype of the patients. Also, in order to choose an appropriate treatment for the patients, knowing the exact mutation is important.

Here we used MLPA method to determine copy number of the exons of Dystrophin gene in 37 unrelated DMD/BMD patients in which the deletions were not detected by multiplex PCR designed for 27 exons and the promoter of dystrophin gene. Fragment separation was performed using a 3130 ABI capillary sequencer and the raw data was analysed by Gene marker software (v1.6).

MLPA enabled us to find deletions and duplications in 4 patients. Also the exact borders of deletions and duplications were determined.

In conclusion the method is accurate and reliable. It makes it possible to identify deletions and duplications of all 79 exons of dystrophin gene in patients. Therefore it provides a better diagnosis compared to the most of the common techniques like multiplex PCR and real-time PCR.

P12.078
Serum Matrix Metalloproteinase-9 (MMP-9) as a promising biomarker for disease progression in Duchenne Muscular Dystrophy (DMD)

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Three proteins [MMP-9, Tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) and Osteopontin, (OPN)] implicated in inflammation, extracellular matrix degradation and tissue fibrosis were selected with the aim to identify serum biomarkers for monitoring the progression and treatment outcome of DMD.

Serum biomarker levels were determined using commercially available immunoassays. We observed serum levels of MMP-9 (median level= 1185.34 ng/ml, IQR= 966.56) and TIMP-1 (median level= 245.55 ng/ml, IQR= 69.57) to be significantly higher in DMD patients (N=63) compared to healthy controls (N=16)(MMP-9 median level= 73.22 ng/ml, IQR= 121.00 and TIMP-1 median level=164.77 ng/ml, IQR= 62.45). OPN levels however showed no significant difference between groups. MMP-9 levels were also significantly higher in older non ambulant (N=34) patients compared to younger ambulant DMD (N=29) patients suggesting an increase in MMP-9 levels with disease progression (ambulant MMP-9 median levels= 927.89ng/ml, IQR= 885.60; non ambulant MMP-9 median levels=1328.45ng/ml, IQR= 822.95). Pearson's correlation analysis confirms significant positive correlation between MMP-9 levels, age and duration of corticosteroid treatment in the 63 DMD patients. However linear mixed model analysis with nine DMD patients whose serum samples were collected longitudinally over four years showed significant and positive linear relationship exists between MMP-9 and age i.e. disease progression, and the duration of steroid treatment did not contribute significantly to this relationship.

In conclusion, we identify MMP-9 as a serum biomarker for DMD disease progression and as such will be useful to monitor response to therapies that are currently in clinical trials.

P12.079
Analysis of the dystrofin gene in Armenian patients

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Duchenne (DMD) and Becker (BMD) muscular dystrophy are allelic disorders caused by mutations in the dystrofin gene. The molecular genetic analysis of these disorders in among the most difficult encountered in a routine laboratory diagnostic. The deletios vary in size and location, but are clustered in three "hot spot", the major site encompassing exons 41-60, and a minor region including exons 3-19. Deletions are detected using a multiplex polymerase chain reaction method, in which 26 exons are analyzed in three separate PCR reactions. These exons were chosen to include the two deletions 'hot spots', and this system is estimated to identify approx 98% of all deletions.

There is no data of this mutation and frequencies of carriers in Armenian population. In 27 armeniam patients with a clinical suspicion of DMD/BMD molecular testing has been performed for 26 exons frequent mutations/deletions. The genotyping analysis of this patients revealed mutations in 10 cases.

The frequent detected mutations are in 3, 49 exons in 11% and 50 in 33% correspondingly. The other mutations were detected in 42-43: 46-47: 6: 13: and 52 exons.

P12.080
Ectodermal dysplasia-syndactyly syndrome (EDSS): broadening the spectrum of *PVRL4* mutations and phenotypic delineation among "nectinopathies"

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Ectodermal Dysplasia-Syndactyly Syndrome (EDSS; OMIM#613573) is a rare autosomal recessive condition with abnormal hair (pili torti), progressive alopecia, teeth dysplasia, and hands/feet cutaneous syndactyly. Recently, we identified PVRL4 mutations as the cause of EDSS in two families from Algeria and Italy. PVRL4 encodes nectin-4, a cell adhesion molecule relevant for cadherin-based adherens junction formation.

Four nectins have been described so far. Notably, PVRL1 (encoding nectin-1) mutations cause Cleft Lip/Palate-Ectodermal Dysplasia Syndrome (CLPED1; OMIM#225060) also known as Zlotogora-Ogur syndrome, suggesting the common term "nectinopathies" for this group of disorders.

We have ascertained a further consanguineous family with EDSS and identified a novel missense alteration in PVRL4. Affected individuals were homozygous for an evolutionary conserved p.Val242Met change (c.724 G>A), that was predicted to be pathogenic by computing analysis. Modelling analysis showed that the p.Val242 residue is part of a linker between the 2nd-3rd Ig-like domain and suggested that the replacement with the larger Met residue imposed new nectin-4 contacts with p.Arg251 and p.Glu273, potentially affecting homo-/heterophilic interactions with other Ig-like molecules.

This family adds to the two previously described easing the delineation of a very homogeneous phenotype resulting from PVRL4 mutations and characterized by: thick and fragile hair with pili torti, hair loss with patchy alopecia starting at childhood and affecting as well eyebrows and eyelashes, conical and abnormally spaced teeth, and partial cutaneous syndactyly of digits and toes (2-3 and 4-5).

Our description of EDSS reveals broad clinical overlap with the PVRL1-associated nectinopathy, with cleft lip/palate being a distinguishing feature.

P12.081
EGFR gene mutation testing in non-small cell lung cancer (comparison of different methods)

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Targeted cancer therapy requires the rapid, sensitive and accurate identification of genetic abnormalities predicting the therapeutic response. Somatic mutations in the epidermal growth factor receptor (EGFR) gene are associated with the objective reponse to EGFR tyrosine kinase inhibitors (TKI). Non-small cell lung cancer (NSCLC) patients harboring EGFR mutations benefit from this specific inhibitors. Almost of all mutations are located within exons 18 - 21 of the EGFR gene (85-90% of the mutations occured at exons 19 and 21 in EGFR gene).

The analysis of EGFR mutations was performed on DNA isolated from cytology sample and formalin-fixed and paraffine-embedded (FFPE) tissues from NSCLC patients.

The aim was comparison of different methods and finding a sensitive, reliable, simple and fast method for routine screening of these mutations in clinical practice, because sometimes is difficult to obtain sufficient tumor sample of patients with NSCLC. EGFR gene mutations were analysed by following methods: direct sequencing, fragment analysis (exon 19 of the EGFR gene), primer extension analysis (SNaPshot assay), real-time PCR (Scorpions-ARMS) and mutant-enriched PCR targeting exons 19 and 21 of EGFR gene. We examined 43 tumor samples of patients with NSCLC.

SNaPshot assay and mutant-enriched PCR, simple and high sensitive assays, were proved as suitable methods for routine mutational analysis of EGFR gene in NSCLC patiens in our laboratory. This applies especially for the cytology samples with only few cancer cell and thus limited amount of DNA.

P12.082***

Loss-of-function mutations of *CHST14* cause a new type of autosomal recessive Ehlers-Danlos syndrome

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Ehlers-Danlos syndrome (EDS) is a heterogeneous connective tissue disorder involving skin and joint laxity and tissue fragility affecting as many as 1 in 5,000 individuals. We reported a novel subtype of EDS similar to kyphoscoliosis type but without lysyl hydroxylase deficiency. Patients are characterized by distinct craniofacial characteristics, multiple congenital contractures, progressive joint and skin laxity, and multisystem fragility-related manifestations. As two of six patients are consanguineous families, we hypothesized this is autosomal recessive disorder. We mapped its locus at 15q15.1 by homozygosity mapping using two consanguineous families and identified a homozygous *CHST14* mutation in the two families and compound heterozygous mutations in four other sporadic cases. Transfection experiments of mutants and enzyme assays using fibroblast lysates of patients showed the loss of D4ST1 activity. *CHST14* mutations altered the glycosaminoglycan (GAG) components in patients' fibroblasts. Interestingly, dermatan sulfate (DS) of decorin proteoglycan, a key regulator of collagen fibril assembly, was completely lost and replaced by chondroitin sulfate (CS) in the patients' fibroblasts, leading to decreased flexibility of GAG chains. The loss of the decorin DS proteoglycan due to *CHST14* mutations may preclude proper collagen bundle formation or maintenance of collagen bundles while the sizes and shapes of collagen fibrils are unchanged as observed in the patients' dermal tissues. These findings indicate the important role of decorin DS in the extracellular matrix and a novel pathomechanism in EDS.

P12.083

Ellis-van Creveld syndrome - a new UKGTN service

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Ellis-van Creveld syndrome (EvC) is an autosomal recessive chondrodysplasia, characterised by short ribs, short limbs, postaxial polydactly, dysplastic nails and teeth and a range of dental anomalies. Congenital heart defects occur in 60% of cases.

EvC is caused by mutations in *EVC* (21 exons) and *EVC2* (22 exons) at 4p16. These genes are divergent in orientation separated by 2.6kb. EvC is a rare disorder most prevalent in the USA Amish. The birth prevalence in non-Amish is estimated as 0.7/100,000. Consanguinity is reported in 30% of cases (Ulucan *et al.* 2008, *BMC Med Genet* **9:** 92).

A new UKGTN service for Ellis-van Creveld has been established using high throughput semi-automated bidirectional sequencing of 44 fragments followed by analysis using Mutation Surveyor (Softgenetics). Sequencing of *EVC/EVC2* is expected to detect approximately 69% of EvC cases (Tompson *et al.* 2007, *Hum Genet* **120:** 663-670). The service has been validated using 17 EvC samples provided through our collaboration with Institute of Human Genetics, Newcastle.

The identification of unclassified variants remains a challenge for diagnostic laboratories. We present service results to date including two interesting cases found to be homozygous for novel variants. One patient of consanguineous parents with an antenatal diagnosis of EvC is homozygous for c.451-1_452del in *EVC2*; this deletion at the intron-exon boundary of exon 4 is predicted to alter the acceptor splice site, resulting in exon skipping. The second patient is homozygous for a c.2507C>A; this results in a premature STOP codon in exon 17 of *EVC*.

P12.084

A novel homozygous mutation of the ENPP1 gene causes generalised arterial calcification in infancy

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Generalized arterial calcification of infancy (GACI, MIM#208000) is a rare autosomal recessive disorder generally due to homozygous/compound mutations of the ENNP1 gene (6q22-q23). Classical features include calcification of large- and medium-sized arteries and marked myointimal proliferation leading to arterial stenosis. Extravascular signs include foci or periarticular calcifications. Most affected children die in the infancy as consequence of vascular occlusion (myocardial infarction) or congestive heart failure due to pulmonary hypertension. We here describe a child diagnosed with GACI harbouring a novel mutation of the ENNP1 gene.

Methods and Results. The proband was born preterm with a respiratory distress; he is a son of two consanguineous Turkish parents. Echocardiography showed increased echogenicity of the walls of pulmonary arteries, the aorta and the coronary arteries, patent foramen and thin pulmonary valve. Additionally, calcification foci have been localised within the aortic arch, thoracic aorta and both carotids. Because of the above features the ENPP1 gene was screened. Bidirectional sequencing of the gene showed a gene re-arrangement (Insertion and Deletion) within the exon 20 in the proband. Both parents were carriers of the above mutation.

Conclusions. This work identified a novel mutation of the ENNP1 gene linked to the GACI disease. When in heterozygous state this mutation leads to mild clinical sign such as short stature, facial dysmporhysms and hypophosphatemic rickets. In the homozygous condition GACI features are present. The identification of a ENPP1 mutation allowed prompt biphosphonate treatment and the establishment of a causative link between the GACI syndrome and the ENPP1 gene.

P12.085

Acid Fatty Metabolism: a new pathway involved in Epidermolysis Bullosa Simplex

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Epidermolysis bullosa simplex (EBS) is the most common form of inherited epidermolysis bullosa. The disease is characterized by intraepidermal blistering due in most cases to mutations in cytokeratin 5 (*KRT5*) or 14 (*KRT14*) genes and patients present with widely varying severity.

In order to document the molecular biology of the disease, we established an EBS biobank including biological materials from six Canadian EBS patients (2 EBS-DM and 4 EBS-loc patients) and six unaffected individuals paired for the age and gender. We first characterized these patients at the genetic level and we identified six pathogenic mutations from which three are novels (I412F (K14), R125S (K14), and R559X (K5)). Then, we performed an expression microarray analysis of the EBS epidermis tissue in order to examine the broad spectrum of effects of *KRT5* and *KRT14* mutations "*in vivo*" and to identify potential regulatory networks and pathways altered in this disease. Expression profiling show that 28 genes are differentially expressed in EBS patients compared to control subjects (p value <0.05 and absolute fold change >2). Nine genes (*AWAT2*, *DGAT2L6*, *ELOVL3*, *THRSP*, *FADS2*, *FAR2*, *ACSBG1*, *AADACL3*, and *CRAT*) are implicated in fatty acid metabolism and three other genes (*SPRR2B*, *SPRR4*, and *KRT79*) in epidermal keratinisation. These two biological pathways contribute both to the formation of the cell envelope barrier structure. This study demonstrates, for the first time, the relevance of metabolic cluster, specifically fatty acid metabolism in EBS biology.

P12.086
Chromosome 15q13.3 region disruptions in idiopathic generalized epilepsy

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We report on two patients with different phenotype of idiopathic generalized epilepsy (IGE) bearing microduplication and microdeletion of the 15q13.3 region, respectively, both inherited form their unaffected mothers.

A 13 years old girl suffering IGE with generalized tonic-clonic seizures was analyzed by MLPA for 15q13.3 deletions/duplications (MLPA kit P297-B1). A microduplication involving only the CHRNA7 gene was found. The molecular-genetic analysis of the family members showed that the mother and the sister carry this microduplication, but they did not report any history or presented symptoms of neurological disturbances. The father was healthy and negative for the microduplication. We also searched for 2 bp deletion polymorphism in the CHRFAM7A fusion gene and it was detected in both parents. The children did not carry this polymorphism. Unfortunately, at present it was not possible to determine the exact duplication breakpoints. The role of 2 bp deletion polymorphism in the CHRFAM7A fusion gene is unclear.

The second patient is a boy with idiopathic childhood absence epilepsy and mild mental retardation. MLPA analysis showed chromosome 15q13.3 deletion, covering [15q13.2 - MTMR15, TRPM1, KLF13, CHRNA7 - 15q13.4] gene probes. The genetic defect was inherited from the patient's mother, who did not report any past or present signs of neurological impairment.

Given the fact, that other similar reports exist and that there is no definite explanation for the phenotypic variability of 15q13.3 region disruptions, one possible hypothesis could be the so called "two-hit model".

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P12.087
Whole-exome sequencing to detect mutations causing light-sensitive epilepsy with jaw spasms in a large family

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Aim: To identify the genetic variant(s) underlying light-sensitive epilepsy with jaw-spasms in a large Dutch family.

Methods: A large family showing strong light sensitivity up to the age of 50, and suffering from jaw-spasms when talking, was collected and described. We performed linkage analysis with a 6000 SNP-array (Illumina) to identify regions linked to the light-sensitivity. In the proband we performed whole-exome sequencing (Agilent/SOLID). Variants under the linkage peak passing filters of being novel, functional and predicted damaging were sequenced with Sanger sequencing in the family to identify segregation patterns. Genes with variants co-segregating with the phenotype are currently followed up by sequencing all exons in healthy controls and in other light-sensitive patients.

Results: The linkage analysis resulted in four linkage peaks with LOD-scores around 2.9. The total sequencing effort identified almost 380 exonic variants under the linkage peaks, of which around 30 were novel, and less ten than were predicted to be functional. An interesting variant was found in *SCNM1*, sodium channel modifier 1, and this variant was co-segregating with the phenotype. Follow-up of the variant, as well as other variants, is in progress.

Conclusions: *SCNM1* is a good positional and functional candidate for the phenotype in this family. Follow-up must shed more light on the probability of this variant as the cause (or one of the causes) of the phenotype in this family.

P12.088
One novel and one recurrent mutation in SCN1A gene

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The range of disease phenotypes produced by mutations in the SCN1A gene is quite broad. SCN1A mutations have been linked to several epilepsy syndromes sharing some common clinical features of different severity and in rare cases of familial migraine and autism. We report on two patients with SCN1A mutations and severe epilepsy within the spectrum of generalized epilepsy with febrile seizures plus syndrome (GEFS+), the phenotypes being consistent with Dravet syndrome (DS) and myoclonic astatic epilepsy (MAE, or Doose syndrome), respectively. Analysis of SCN1A revealed a heterozygous de novo frameshift mutation (c.4205_4208delGAAA) in the patient with DS, and a recurrent missense mutation (c.3521C>G) in that suffering from MAE. The missense mutation has been reported in patients with neuronal diseases of various manifestations, which suggests that this variability is likely to result from the modifying effects of other genetic or environmental factors. To date, truncation mutations have been found exclusively in patients with DS, while manly missense mutations and very few nonsense mutations have been found in the milder GEFS+ phenotypes.

P12.089
STXBP1-related encephalopathy manifesting as infantile spasms and generalized tremor in three patients

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Twenty-four patients with dominant mutations in the *STXBP1* gene causing epileptic encephalopathy have been published. We report on three patients with *STXBP1* mutation/deletion. Two patients belong to a series of 29 patients with infantile epileptic encephalopathy selected for a sequencing of *STXBP1*. Identified *de novo* mutations were the recurrent p.Arg406His substitution and an insertion of six bases inducing a frameshift. The third patient was diagnosed with a deletion of *STXBP1* by CGH-array. All three patients had infantile spasms associated with partial seizures that responded to antiepileptic drug therapy, severe intellectual impairment, and a striking tremor.

Mutations in STXBP1 are relatively frequent in patients with infantile epileptic encephalopathies. STXBP1-related encephalopathy may present as drug-responsive infantile spasms with focal/lateralized discharges. Generalized tremor may be a clue to the diagnosis in some patients.

P12.090
Sorting defects of mutant CLC-Kb in Bartter syndrome.

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Bartter syndrome is an autosomal recessive disorder characterized by renal salt wasting and hypokalaemic metabolic alkalosis. Bartter syndrome type 3 is caused by mutations in *CLCNKB*, encoding the renal chloride channel CLC-Kb. Defects of the *CLCNKB* variants may include a disturbed subcellular sorting or defective gating. Analysis of these variants in polarized epithelial cells is a valuable tool to assess their sorting properties. Here, we present the analysis of the subcellular distribution of eleven CLC-Kb missense variants in such cells.

MDCK type II cells were transfected with GFP-tagged CLC-Kb encoding constructs and subjected to imunnofluorescence studies and confocal laser-scanning microscopy. Of the eleven variants, five (S113T, A204T, M427V, G465R and R595Q) were transported to the basolateral plasma membrane, like wt-CLC-Kb. Five others (P124L, G164R V170M, G433E en L656P) were retained intracellularly, and colocalized to a major extend with an endoplasmic reticulum (ER) marker or vesicles (only V170M). The final variant (C626Y) showed a unexpected staining of both the nucleus and the basolateral plasma membrane.

These data indicate that a subset of the CLC-Kb variants is properly sorted to the basolateral plasma membrane. These variants may reflect non-pathogenic variants, or pathogenic variants that are functionally impaired (i.e. defective gating). However, another subset is retained in the ER, as is common with misfolded membrane proteins, such as CFTR or AQP2. One mutant (V170M) shows a yet to be determined vesicular staining. Altogether, studying the subcellular distribution of CLC-Kb variants in polarized cells is a valuable additive tool to test their pathogenicity.

P12.091
Facioscapulohumeral Muscular Dystrophy (FSHD1) - Simple Sequence Length Polymorphisms (SSLP) haplotype analysis which confirms pathogenicity on ‚permissive' chromosomal backgrounds.

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FSHD (FSHD1; OMIM 158900) is an AD disease affecting 1 in 20,000 and is the third most common form of muscular dystrophy, characterized by progressive wasting of upper body muscles. FSHD1 is associated with a contraction of D4Z4 tandem repeat units in the subtelomere region 4q35. Diagnosis is complicated by the homologous nature of 4q and 10q D4Z4 arrays.

Bristol Genetics laboratory offers a UKGTN specialist diagnostic FSHD service for UKGTN and international laboratories and processes around 400 referrals annually for diagnostic, predictive, and prenatal testing. Molecular confirmation of FSHD1 is currently by EcoRI/BlnI/ApoI digests using probe p13E-11 (to confirm the chromosome of origin and size of the D4Z4 contraction), and distal 4qA/4qB haplotyping (4qA being associated with the pathogenic haplotype). Patients with a deletion of the p13E-11 region (~2%) can be identified using the D4Z4 1kb probe.

Lemmers et al (Science 2010; 329(5999):1650-3) has recently published a unifying genetic model for FSHD. FSHD patients were reported to carry specific single nucleotide polymorphisms (SNPs) distal to the last D4Z4 repeat, which create a polyadenylation site for transcripts derived from DUX4. Specific SNPs are suggested to result in efficient polyadenylation and increase DUX4 transcript stability thereby causing FSHD through a toxic gain of function. In FSHD1 the contraction of the D4Z4 repeat units is only pathogenic in 4 specific 'permissive' chromosomal backgrounds 4A161, 4A161L, 4A159 and 4A168.

We present cases and data for establishing SSLP haplotype analysis as an additional diagnostic service for FSHD1.

P12.092
Genetic Peculiarities of Juvenile Chronic Arthritis in patients with Familial mediterranean fever.

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Familial Mediterranean fever (FMF) is the most common autosomal recessive autoinflammation disorder in populations of Mediterranean region ancestors. FMF is caused by MEFV gene mutations (homozygous and compound heterozygous genotypes). The frequency of the carriers of MEFV mutations is 1:3 in non-FMF Armenian population. Number of FMF and related disorders is very high in Armenians, formed as a founder population experienced genetic drift and social and geographical isolation. Clinical and molecular investigations in more than 16000 FMF patients demonstrated significant correlation between MEFV-genotypes and phenotype. Familial study showed the transmission of mutations in parents and siblings. Comparing the MEFV genotypes in two generations among the families was found.

Acute recurrent/chronic destructive arthritis is one of the common features of FMF and could be the first, single manifestation. We present the peculiarities of JCA (EULAR criterion) in FMF patients with different spectrum of *MEFV* mutations. *HLA B27* was identified in 58%. JCA manifested first in 58% of patients; FMF joined as short-term rare attacks of thoracalgia or isolated fever and/or hemorrhagic vasculitis with fever. In 42% of patients FMF developed in 2-3 years. In 33% of patients with atypical FMF athropathy and polyserositis was revealed. Patients with JCA should be investigated for familial anamnesis for FMF and *MEFV* mutations. In JCA patients early detection of *MEFV* mutations is important for on-time colchicinotherapy, improvement of arthritic syndrome and prevention of FMF complications. At the same time FMF patients with resistancy to treatment of arthritis should be tested for *HLA-B27.*

P12.093
Comparative analyses of the Fanconi anemia core complex genes reveal conserved transcription regulatory elements

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The Fanconi anemia (FA) gene family is a recent addition to the complex network of proteins that respond to and repair certain types of DNA damage in the human genome. Since little is known about the regulation of this novel group of genes at the DNA level, we characterized the promoters of eight genes (FANCA, B, C, E, F, G, L and M) that assemble into the FA core complex. The promoters of these genes show characteristic attributes of housekeeping genes: high GC content, rich in CpG islands, and lack of TATA boxes. They function in a monodirectional way and are, in their most active portions, comparable in strength to the SV40 promoter in the reporter plasmids. They are marked by a distinctive transcriptional start site (TSS). In the 5' region of each promoter, we identified a region that, in isolation, is able to negatively regulate promoter activity in HeLa and HEK 293 cells. The central and 3' regions of the promoter sequences harbor binding sites for several common and rare transcription factors including STAT, SMAD, E2F, AP1 and YY1, indicating that there are cross-connections to several established regulatory pathways. Electrophoretic mobility shift assays and siRNA experiments confirmed shared regulatory responses between prominent members of the TGF-beta and JAK/STAT pathway and members of the FA core complex. We identified a bi-partite nature to these promoters and the results supported the hypothesis based upon a co-evolution of the FA core complex genes and was expanded to include their promoters.

P12.094
DNA bank of the polyposis syndroms in Poland

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Nowadays molecular genetics is inseparable part of classical medicine to proper diagnostics. Here we present Polish register of patients with the predisposition towards intestinal polyposis. The creation of the register is a result of cooperation between the many oncological, surgical and genetic centers in Poland. Bank comprises the total of 1097 DNA samples derived from 449 families with intestinal polyposis of which 945 samples come from persons in whose families Familial Adenomatous Polyposis (FAP) occurred. We collected the DNA samples from 30 Peutz-Jeghers families, 28 with juvenile polyposis families, 2 Cowden syndrome families and one Proteus syndrome case. Investigations were conducted on DNA isolated from cells of the peripheral blood. The search for mutations in APC, MUTYH , PTEN , BMPR1A, SMAD4 and STK11 genes preconditioning the occurrence of individual diseases was performed employing PCR-SSCP, PCR-HD, DHPLC as well as RFLP techniques and DNA sequencing. General the performed molecular investigations allowed identification of mutations in studied syndromes ranging from 44 to 50%. The mutations spectrum for the genes conditioning polypsis syndromes in Poland was established. The performed investigations also broadened our knowledge about correlations between the genotype and phenotype as well as cases of an atypical course of the disease.

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P12.095

Mutations in FHL1 impair expression and functionality of Kv1.5 in XMPMA patient myoblasts.

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Four-and-a-half LIM domain protein 1 (FHL1), predominantly expressed in skeletal and cardiac muscle, is suggested to play a role in sarcomere synthesis and assembly. Mutations in the FHL1 gene (leading to different FHL1 splice variants) were identified as the cause of different myopathies including X-linked myopathy with postural muscle atrophy (XMPMA). As FHL1 has been addressed to act as a likely modulator of human atrial potassium voltage-gated channel Kv1.5, we here describe physical and functional interaction between FHL1 and Kv1.5 in XMPMA patient myoblasts.

RT-PCR and Western blot techniques revealed almost complete absence of FHL1A and reduction/absence of Kv1.5 in myoblasts from XMPMA patients. Myoblast cell proliferation was decreased in patients compared to controls while an increased number of patient myoblasts was found to reside in the G0/G1 phase. Two-electrode voltage clamp experiments demonstrated that Xenopus laevis oocytes overexpressing Kv1.5 and FHL1 variants exerted markedly reduced currents when compared to oocytes expressing Kv1.5 only. The most pronounced effect was observed for FHL1C. Potential interaction between FHL1C and Kv1.5 was confirmed by a pull down assay. Immunohistochemistry confirmed colocalization of both proteins after overexpression in an atrial cardiomyocyte cell line.

We here describe functional interactions between FHL1 and Kv1.5 in myoblasts from XMPMA patients. Since Kv1.5 expression and activity is altered in XMPMA patient myoblasts it is likely to assume that FHL1 will regulate Kv1.5 channel activity in both cardiac and skeletal muscle leading to (patho)physiological alterations in XMPMA patients.

P12.096

The role of the R92Q *TNFRSF1A* mutation in patients with familial Mediterranean fever

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Objective: To define the frequency of the R92Q Tumor Necrosis Factor Receptor-Associated Periodic Syndrome (TRAPS) mutation in patients with familial Mediterranean fever (FMF) and to study the role of this mutation in FMF.

Methods: Ninety two FMF patients and 250 controls were genotyped for the R92Q mutation. The frequency of R92Q was assessed among 5 groups of FMF patients.

Results: R92Q was found in 6% of the controls, with an especially high carrier rate among Moroccan Jews (8%). R92Q was found in 3 of the 92 FMF patients (3.2%), 1 homozygous for the MEFV M694V mutation and 2 heterozygous for M694V. All 3 patients showed partial response to colchicine. R92Q was not found in patients unresponsive to colchicine, nor was it found in patients with amyloidosis or in patients with FMF like disease without MEFV mutations.

Conclusion: The frequency of the R92Q mutation in FMF patients is comparable to that of controls. Despite the fact that TRAPS and FMF share common biochemical pathways we found no evidence for an interaction between these two genes.

P12.097

Advances in understanding the molecular mechanism of Goldberg-Shprintzen Syndrome

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Goldberg-Shprintzen syndrome (GOSHS, MIM #609460) is an autosomal recessive disorder associating severe intellectual disability, typical facial gestalt and Hirschsprung disease. KIAA1279 was identified as the disease causing gene with homozygous nonsense mutations. The encoded protein KBP (KIF-binding protein) was initially claimed to be located at the mitochondria, a subcellular localization not confirmed by a recent study. To assess the functional consequences of the KIAA1279 truncating mutations, degradation of the relevant transcripts by the nonsense mediated mRNA decay (NMD) was tested. Finally, because of the putative localization of the protein to the mitochondria, we also studied the effect of this mutation on the mitochondrial function.

We report two novel patients diagnosed with GOSHS, and homozygous truncating KIAA1279 mutations. In fibroblasts of one of the patients and his father (heterozygous) we quantified the KIAA1279 mRNA by RT-qPCR and the protein by Western blot, studied the subcellular localization by immunocytochemistry and the activities of respiratory chain complexes by spectrophotometry.

KIAA1279 mRNA was barely detectable in the patient's fibroblasts and a two-fold decreased was observed in the father's fibroblasts as compared to control, suggesting a NMD mechanism. KBP protein quantification confirmed these results. The activities of the respiratory chain complexes were normal in the patient's fibroblasts.

In conclusion, we report for the first time a KIAA1279 NMD as the molecular mechanism of GOSHS. The absence of normal KBP protein did not influence mitochondrial respiratory chain complex activity in human fibroblasts.

P12.098
Identification of Alu-mediated, large homozygous deletion of *HAX1* gene spanning exons 4-7 in a patient with severe congenital neutropenia

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Severe congenital neutropenia (SCN) is a rare primary immunodeficiency syndrome characterized by sustained neutropenia and early onset of severe bacterial infections. We report a case of a large homozygous *HAX1* deletion in a patient with autosomal-recessive SCN (Kostmann syndrome). The patient was a 2-year old boy who manifested with recurrent bacterial infections at three months of age. The parents and the older sister had no signs of SCN. While performing electrophoretic gel analysis of PCR-products of each exon of *HAX1* gene, we found an absence of PCR-products corresponding to exons 4 through 7 in the proband. For detection of the deletion, a genome-walking method with Universal Genome Walker Kit according to the manufacturer's instructions (Clontech) was used. Amplified products from the nested PCR were cloned using p-GEM®-T Vector System (Promega) and directly sequenced. We identified a homozygous gross deletion in the *HAX1* gene: NG_007369.1:g.(6484_6510)_(8526_8552)del2042. The analysis of the *HAX1* gene revealed that both 5' and 3' breakpoints were located inside Alu elements. Sequence alignment of the hybrid intron and normal introns 3 and 7 indicated recombination between these Alu elements. Each of the unaffected parents of the patient had the same deletion in a heterozygous state. The deletion was not found in a group of 100 healthy controls. Alu-element regions are known to be hot spots for recombination events. Gene rearrangements by Alu-mediated recombination have been reported previously as the molecular basis of some congenital disorders; SCN may now be considered as another example.

P12.099
Identification of a novel deletion in GATA3 gene in a French patient with HDR syndrome

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HDR syndrome is a rare autosomal dominant congenital disorder characterized by hypoparathyroidism, sensorineural deafness, and renal dysplasia. This triad of symptoms does not occur in all patients. Indeed, renal abnormalities are heterogeneous with variable penetrance. HDR syndrome is caused by mutations in the GATA3 gene that belongs to a family of dual zinc-finger transcription factors that play a critical role in embryonic development. So far, various HDR-associated GATA3 mutations occurring throughout the gene have been reported.

Here, we report clinical and molecular findings in 2 French patients with heterozygous intragenic GATA3 mutations. The first patient is a 5 year-old boy. He was diagnosed with PTH-deficient hypoparathyroidism, bilateral sensorineural deafness and vesicoureteric reflux. Direct sequencing of GATA3 identified a duplication occurring in codon 145 (c.431dup), previously described. This frameshift mutation generates a premature stop codon at codon 303, and results in the loss of the nuclear localization signal of GATA3. The second patient is a 15 year-old male and was born with upper eyelid agenesis. He was diagnosed as having hypoparathyroidism, bilateral sensorineural deafness and kidney hypoplasia. Sequence analysis of GATA3 demonstrated a novel deletion c. 807_812del (p.Ser271_Thr272del) in the N- terminal zinc finger1 (ZnF1) of GATA3. ZnF1 is essential to stabilize the C-terminal zinc finger2 (ZnF2) DNA binding, and interacts with other zinc finger proteins, such as the friends of GATA (FOG).

In conclusion, we have identified a novel deletion of the GATA3 gene, associated with HDR syndrome, widening the spectrum of GATA3 mutations that cause this disease.

P12.100
A multiplex ligation-dependent probe amplification (MLPA) assay specifically developed to detect novel deletions causing non-syndromic hearing impairment at the DFNB1 locus

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DFNB1 deafness, caused by mutations in the *GJB2* gene (connexin-26) on 13q12, is the most frequent subtype of autosomal recessive non-syndromic hearing impairment. Molecular testing for *GJB2* mutations is the standard diagnostic approach for subjects with this disorder. However, in 10-50% of affected subjects with *GJB2* mutations, only one mutant allele is found. Some of these unelucidated cases are just coincidental carriers of the most frequent *GJB2* mutations, but a variable proportion of cases across different populations carry in *trans* one of four different deletions at the DFNB1 locus. The number of *GJB2* heterozygotes that remain unelucidated after screening for the known deletions indicates that still unidentified DFNB1 mutations exist. It was hypothesized that all these deletions remove a cis-acting regulatory element that would control *GJB2* expression in the inner ear. Overlapping of all deletion intervals defined a 95.4 kb interval located more than 100 kb upstream from *GJB2* that must contain the regulatory element. To detect further DFNB1 deletions, we designed a multiplex ligation-dependent probe amplification (MLPA) assay featuring 9 probes evenly spaced across the critical interval. By using this assay, we screened a cohort of 236 unelucidated *GJB2* heterozygotes from Spain, the Netherlands, Brazil and Poland. We just identified one Polish subject harbouring the del(*GJB6*-D13S1854) allele, which had not been previously tested for in the entire Polish cohort. Our results imply that other deletions at the DFNB1 locus may be rare or that such deletions would be small enough (<10 kb) to escape detection by this MLPA assay.

P12.101
An overview of Mitochondrial Genetics in Hearing Loss : Research Progress in Iran

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Hereditary hearing loss (HL) is a heterogeneous trait. Mitochondrial DNA mutations are responsible for less than one percent of pre-lingual HL. Causative mtDNA mutations have been found in five percent patients with post-lingual non-syndromic HL . In this regard, mutations in 12S rRNA, RNA ser(UCN) and tRNA Leu(UUR) genes account for most cases of maternally inherited nonsyndromic HL .

In this research, one thousand Iranian HL subjects were included from 10 provinces of Iran . We have screened three common mtDNA mutations including A1555G , A3243G, and A7445G using PCR-RFLP technique. Altogether, 13 different mutation were identified. These mutations were as followed: A1555G (in 5 cases), A3243G (1 case),

G3316A (6 cases), and A7445G (1 case). Interestingly, the prevalence of common mtDNA mutations were different among different Iranian populations .
While a relatively high levels of A1555G were found in Azarbaijan Sharghi (4.91 percent) and Booshehr provinces (4.35 percent in post-lingual non-syndromic HL subjects, respectively. Other mtDNA mutations were detected in much less frequencies in other provinces . In general, mtDNA mutations are present in less than one percent of the children with pre-lingual HL. However, they are found in about 5 percent of children with post-lingual HL in Iran . Our results indicate that mtDNA mutations may play a more important role in maternally inherited non-syndromic post-lingual HL than in the pre-lingual cases in some Iranian populations. We believe, the findings must be considered in the genetic counseling of hereditary HL.

P12.102
Spectrum of mutations in majority of patients with hemophilia A in Slovenia

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The high mutational heterogeneity of hemophilia A is a challenge for provision of genetic services. Our aim was to create a confidential national database of mutations for improvement of genetic services in Slovenia. The factor VIII gene (F8) was analyzed in 139 of 178 hemophilia A patients. The mutations were identified by testing inversions of intron 22 and 1, sequencing part of the promoter, whole coding region and exon/intron boundaries of the F8 gene. There are 178 hemophilia A patients from 126 families in Slovene registry for Hemophilia. We determined mutations in 81/82 patients with severe, 14/16 with moderate and 44/60 with mild form of hemophilia A. The detection rate was 100%. In the Slovene population 40/139 have inversion of intron 22 (48,8% of severe cases) and another 99/139 have 52 different mutations in F8 gene. Of these, 16 are so far found only among Slovene patients. Inversion of intron 1 was not detected in Slovene population of hemophilia A patients. Interestingly, there were no large deletions and insertions. 30% of the small deletions and insertions occurred at stretch of adenines, codons 1191-1194 (8As). A T insertion in exon 26 extends FVIII protein by frame-shift mutation. The spectrum of mutations Slovenian hemophilia A patients was comparable to that found in the Italian and Austrian population, but differed from the spectrum in UK. The type of mutation is a predictor of clinical phenotype. The database is a powerful tool for genetic counseling and medical care of families with hemophilia in Slovenia.

P12.103
The Turkish Hereditary Angioedema Pilot Study (TURHAPS): The First Turkish Series of Hereditary Angioedema

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Background: No published genetic data presently exist concerning hereditary angioedema (HAE) in Turkey. The aim of the present study was to initiate a preliminary multi-centric evaluation about HAE and to identify the causative mutations in a group of patients with hereditary angioedema type I.
Methods: 10 patients with clinical diagnosis of HAE type I were sampled to analyze the entire coding and un-coding exons of *C1NH* gene for mutations by DNA sequencing. Identified new mutations were further evaluated by segregation with the first-degree relatives.
Results: Causative mutations were identified in 9 out of 10 patients, Three of the identified mutations were previously described while 6 were being first time described here in this study. One family showed autosomal recessive inheritance with a novel homozygous mutation , which is a very rare condition as being only one family described in the literature before. Others all had heterozygous mutations pointing out the autosomal dominant inheritance as anticipated. Mutations were found to occur *de novo* in 2 patients while in other 2 the occurrence could not be defined since the parents' DNA were not available. Causative mutation could not be identified in one patient.**Conclusion:**The identified mutations were found to be scattered in all of the exons except 4. It is observed that patients carrying non sense mutations had close to 90% decrease in their C1 esterase inhibitor levels. We hope that our findings in Turkish HAE patients will further support the clinical and molecular data underlying hereditary angioedema

P12.104
ACVRL1 germinal mosaicism with two mutant alleles in a family with Hereditary Haemorrhagic Telangiectasia associated with PAH

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Germline mutations in genes encoding members of the TGF-beta/BMP superfamily are causal for two hereditary vascular disorders, hereditary hemorrhagic telangiectasia (HHT) and heritable pulmonary arterial hypertension (PAH). When the two diseases co-exist, Activin A receptor type II-like kinase-1 (ACVRL1, also known as ALK1) mutations are mainly involved. We report a remarkable ACVRL1 germinal mosaicism characterized by the presence of two mutant alleles in a woman initially diagnosed with PAH at age 21. She also met the Curaçao diagnostic criteria for HHT based on additional findings of telangiectases and epistaxis. Mutation analysis of ACVRL1 identified two closely located heterozygous deleterious mutations within exon 10: c.1388del (p.Gly463fsX2) and c.1390del (p.Leu464X). The two mutations were transmitted independently to the three sons of the index case. One son received the c.1388delG and two sons received the c.1390del. They all present symptoms of HHT, with various intensity, which needs to be interpreted with regards to their young age. Allele-specific PCR analysis demonstrated that c.1388del is the predominant mutation in lymphocytes of the index case. Haplotype analysis revealed that both mutant alleles have a common origin. The presence of these two distinct alterations might result of an incorrect repair of a larger de novo mutational event that occurred in the early stage embryo. An alternative hypothesis is that these two distinct alterations might be explained by a revertant mosaicism mechanism.

P12.105
Amplicon based high-throughput, pooled sequencing identifies mutations in CYP7B1 and Paraplegin in AR-HSP

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Hereditary spastic paraplegia (HSP) is a neurodegenerative disorder defined clinically by progressive lower limb spasticity and weakness. HSP is a highly heterogeneous condition with at least 48 loci identified so far, involving X-linked (XL), autosomal recessive (AR) and autosomal dominant (AD) inheritance. For correct diagnosis molecular testing is essential since clinical parameters by themselves are not reliable to differentiate HSP forms. Thus, the purpose of this study was to establish amplicon based high throughput genotyping for AR-HSP. A sample of 187 index cases with sporadic or recessive spastic paraplegia were analyzed by applying an array based amplification strategy to generate amplicon libraries followed by a pooled next-generation sequencing (NGS) approach to investigate the CYP7B1-(SPG5) and Paraplegin-gene (SPG7). We identified one SPG5 and four SPG7 patients. All of them were compound heterozygous for two mutations. In total, three known CYP7B1 mutations and eleven mutations in Paraplegin, including five novel mutations (p.W29X, p.R139X, p.R247X, p.G344D and p.R398X), were detected through amplicon-based next generation sequencing. Discovering the molecular basis of the neurodegenerative disorder HSP is challenging given the increasing number of subtypes. Our study illustrates how amplicon based NGS can be used as a suitable platform to provide data on molecular mutations at a necessary throughput and accuracy.

P12.106
Homozygosity mapping of an Omani family affected with hereditary Spastic paraplegia overlaps with SPG24 locus

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Hereditary spastic paraplegia (HSP) is a group of neurodegenerative diseases genetically inherited from one generation to another. About thirty-four different loci have been mapped so far, among them 16 genes have been identified. Autosomal recessive forms of spastic paraplegias usually have clinically complex phenotypes but the SPG5, SPG24 and SPG28 loci are considered to be associated with pure forms of the disease. Stevanin G*, et al 2009*.

Two affected boys from a consanguineous family were diagnosed with HSP. The parents have two other kids a boy and a girl and both are normal. The symptoms were reported to start as early as six months of age. In one of them, spasticity is associated with epilepsy. The MRI showed abnormality in the brain.

The genomic DNA was typed for SNP markers in 10K SNP chip microarray using Affymetrix platform. The genotypes were viewed and sorted according to their chromosomal location and to their physical position in an Excel file. The homozygosity regions were determined using simple algorithm in Excel. Those regions were compared against known HSP loci in the literature to find out if any one of them is actually causing the disease.

The second largest region of homozygosity overlapped with a reported HSP locus, which is SPG24 identified by Hodgkinson et al (2002). No gene has been identified in the region yet. Although SPG24 have been reported as pure HSP but the symptoms in our case is associated with epilepsy and MRI changes. We are currently perusing candidate gene sequencing.

P12.107
Molecular diagnosis of hereditary neuropathy with liability to pressure palsies

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Hereditary neuropathy with liability to pressure palsies (HNPP) is an autosomal-dominant disease. Motor and sensory nerve dysfunction due to compression at common entrapment sites is the characteristic clinical presentation of this disease, even though there is a wide clinical spectrum of presentation, including asymptomatic individuals HNPP electrophysiological findings show a distinctive background polyneuropathy, independent of superimposed entrapment neuropathy. It is characterized by diffuse sensory nerve conduction velocity (SNCV) slowing and prolongation of distal motor latencies with relatively infrequent and minor reduction of motor nerve conduction velocities. This indicates disproportionate distal conduction slowing in the disorder.

Up to 84% of HNPP are due to the 1.5 Mb deletion at 17p11.2-12 containing the *PMP22* gene. This gene codifies for the membrane glycoprotein, Peripheral Myelin Protein 22, which plays an important role in the formation and maintenance of compact myelin in the peripheral nervous system. Small deletions in PMP22 (detected with microsatellite analysis or Multiplex ligation-dependent probe amplification studies), point mutations in the *PMP22* gene, point mutations at MPZ have been reported to cause HNPP. In addition, mutations in connexin32 gene present as a neuropathy with intermediate NCV that could mimicry HNPP. We propose a HNPP diagnosis algorithm including (microsatellite analysis, MLPA and direct sequencing analysis of PMP22, MPZ and Connexin32 gene) that allows the diagnosis of almost 90% of suspected HNPP patients.

P12.108***
Apolipoprotein B is a new target of the RET/GDNF signalling pathway

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RET and glial cell line-derived neurotrophic factor (GDNF) regulate cell survival, differentiation, migration and proliferation of neural crest-derived cells. Many signalling mediators of RET have been characterized but the activated downstream genes at the end of the signalling cascade initiated by RET are largely unknown. Since the molecular basis of the interaction between RET and EDNRB (endothelin receptor type B) has been characterized, we used a Caenorhabditis elegans knock out strain of nep-1, homologue of human ECE1 (endothelin-converting enzyme-1) to identify new downstream genes.

Transcriptome comparison between wild-type and nep-1 strains identified vit-3 as a differentially expressed gene. We confirmed that Apolipoprotein b, the mammalian gene homologue of vit-3, is activated by the RET/GDNF signalling pathway in Neuro2a cells via the MAPK P38 kinase and it is inhibited by p53. We demonstrated that RET/GDNF and EDNRB/endothelin 3 (ET-3) cooperate in inducing neuronal differentiation resulting in Apob activation. In addition, ApoB expression is downregulated in mouse embryos homozygous for the mutation ret C620R, causing HSCR in humans, whereas the heterozygous mice show a significant increase in ApoB expression and do not show any HSCR phenotype, suggesting that ApoB has a significant role in ENS development.

The new finding that Apob is a downstream signalling target of RET opens up a new perspective in studying RET roles in human health and diseases.

P12.109
A study of the Huntington´s disease associated trinucleotide repeat in Slovak population

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Huntington´s disease (HD) is a progressive neurodegenerative disorder, which is clinically characterized by motor dysfunction, cognitive impairments and psychiatric disturbances. The molecular basis of the disease is the expansion of the trinucleotide CAG in the first exon of the huntingtin gene in chromosome 4p16.3. Diagnosis of HD has been greatly simplified by the direct triplet repeat gene test. In the Prešov region (1998-2009) CAG repeat lenghts in the HD gene were tested in twelve patients with suspected diagnosis of HD. Genomic DNA was extracted from blood samples by standard extraction procedures using Jet Quick® (Genomed). Thermal cycling of PCR conditions were 95°C for three minutes, followed by 30 cycles of 94°C for one minute, 62°C for one minute, 72°C for one minute, with a final extension at 72°C for seven minutes performed in a the thermal cycler Biometra®. PCR products were resolved on capillary electrophoresis with ABI technology. In seven affected patients clinical diagnosis of HD was confirmed by molecular-genetic methods. The sex ratio in the cases, in which the diagnosis of HD has been confirmed was 4:3 in favor of female individuals. Mapping the occurance of HD in the Prešov region is a part of population-genetic analyses and monitoring of the Prešov region population health status.

P12.110***
SCA2 normal allele modifies severity in Huntington disease

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Huntington disease (HD) is a dominantly inherited disorder, caused by a CAG repeat expansion in HTT gene resulting in a extended polyglutamine (polyQ) tract. Approximately 50-70% of the variability in age at onset is due to the CAG repeat length, with the remainder likely to be due to modifying genes. Hypothesizing the potentially additive toxic effect of other polyglutamine proteins, we investigated their influence on the age at onset and severity in HD.
We analyzed the length of the polymorphic polyQ stretches in 10 genes in 216 HD patients and 71 controls: HTT, DRPLA, JPH3, AR, SCA1, 2, 3, 6, 7, 17. Linear regression analysis was applied to assess the linear relation of the age at onset depending on the expanded CAG repeat length. Severity was quantified by dividing the motor score by disease duration.
As expected the HTT longer allele was significantly associated with age at onset ($p<0.0001$), but not the size of the shorter allele, nor their interaction. In addition, DRPLA longer and shorter alleles were significantly associated with age at onset variability ($p=0.0092$ and 0.0013, respectively) as well as their interaction ($p=0.0017$). The expansion length in the other genes tested did not modulate the age at onset in HD. However, disease severity was influenced by the size of the CAG repeat expansion in SCA2 ($p<0.03$).
In addition to the pathological HD expansion, normal CAG repeat lengths in DRPLA and SCA2 genes influenced significantly the age at onset and severity in HD, respectively.

P12.111
The contribution of the self PolyQ load [somatic mosaicism] in the CNS to the onset, disease duration and progression rate of SCA2.

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Introduction: SCA2 show strong relationship among CAG and the onset of ataxia. Other factors might accounts for the onset and other phenotypic features. The best modifier might be intrinsically depending/causing of the unstable nature of the CAG. Aims: 1) To compare the somatic mosaicism of the expanded CAG of SCA2 gene in CNS. 2) To determine the influence of somatic mosaicism on SCA2 phenotype and its relationship with CAG size and architecture and haplotype. 3) To gain insights about the dynamic of the CAG expansion in CNS of SCA2. Methods: We have analyzed CAG expansions in 12 different sites of SCA2 deceased patients with discordant phenotypes and somatic mosaicism indices, peaks number, CAG range and skewness of the CAG in each region was determined. Also, detailed clinical data using rating scales trough life with follow-up using neurophysiology biomarkers were used to generate phenotypic profiles. Results: Regions of the brain with greatest level of somatic mosaicism were motor cortex, occipital grey matter, olive, pons, and globus pallidus. While those regions more compromised in SCA2, like cerebellar cortex showed lesser somatic mosaicism. Early onset was associated with wide ranges of CAG in the CNS (with differences up to 10-17 CAG units respecting the major CAG) in contrast to delayed onsets. Sequence and STR haplotype altogether with phenotypic data are also presented. Conclusions: Our results bring data about the role of the somatic mosaicism as the major modifier of the SCA2 phenotype.

P12.112
The molecular diagnostics of familial hypercholesterolemia in Czech Republic

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Familial Hypercholesterolemia (FH), a major risk factor for coronary heart disease (CHD), is autosomal dominant disorder associated with mutations in the genes encoding the low-density lipoprotein receptor (LDLR), apolipoprotein B100 (APOB), and proprotein convertase subtilisin/kexin type 9 (PCSK9). Predominantly, the clinical phenotype of FH is caused by mutations in the LDLR or APOB genes.
The frequency of heterozygotes is 1/500. The frequency of homozygotes or compound heterozygotes is 1/1 000 000. The high LDL-cholesterol level frequently gives rise to tendon and skin xanthomas, arcus corneae, and accelerated atherosclerosis resulting from cholesterol deposition in the arterial wall, thereby increasing the risk of premature CHD. The identification and treatment of FH patients and their affected relatives with effective lipid-lowering agents is important as this has been shown to significantly reduce both coronary morbidity and mortality.
Mutations in the LDLR and APOB genes were determined using exon by exon screening methods based on individual exon amplifications, restriction analysis, DHPLC, sequencing, and MLPA.
In the set of 2340 probands with the clinical diagnosis of FH, we detected 311 patients (13.3%) with the APOB mutation p.Arg3527Gln and 606 patients (25.9%) with a LDLR mutation. In 606 probands carrying a LDLR mutation, 133 unique allelic variants were detected: 69.9% of these variants were DNA substitutions, 16.5% small DNA rearrangements, and 13.5% large DNA rearrangements. Sixty-three variants were new, not described in other FH populations.
This work was supported by grant MSMT 2B08060 and LC06023.

P12.113
Mutations in *WNT10A* account for 26% of patients with an-/hypohidrotic ectodermal dysplasia

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Hypohidrotic ectodermal dysplasia (HED) is a rare genodermatosis, affecting ectoderm-derived structures, like teeth, sweatglands, nails and hair. The condition can be caused by mutations in the X-linked *EDA* gene, or the autosomal *EDAR, EDARADD,* and *WNT10A* genes. We performed mutation scanning in index cases from 124 families from the Netherlands and Belgium who sought genetic counseling. Mutations in *EDA* were detected in 51% of the index cases, 26% had mutations in the *WNT10A* gene, 6% in the *EDAR* gene, and no pathogenic mutations were detected in *EDARADD*. These data are comparable to those published by Cluzeau et al (2011) in a smaller group of patients from France. Involvement of *WNT10A* has only been established recently. The inheritance pattern of *WNT10A* mutations is not clearly recessive, with some relatively severly affected individuals carrying only one mutation. We will present genotype-phenotype correlations in our group of patients and their relatives. Index cases either carry two nonsense mutations, one nonsense and one missense mutation, two missense mutations or only one missense mutation. Four missense mutations were novel. Notably, one of our patients had pili torti. It appears that a heterozygous nonsense mutation is not enough to cause *WNT10A* related ectodermal dysplasia, since none of the index cases carry only one nonsense mutation and relatives carrying one nonsense mutation are not or only very mildly affected. There is variable expression between relatives with the same genotype. The collection of clinical data from index cases and their heterozygous relatives is ongoing.

P12.114
Homozygousity mapping in a large Iranian pedigree affected with Autosomal Recessive Congenital Ichthyosis(ARCI) reveals linkage to region encompassing NIPAL4/Ichthyn

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Ichthyosis is a genetically and clinically heterogeneous group of cornification disorders characterized by scaling of whole body skin. A subgroup of ichthyosis (ARCI) exhibits autosomal recessive inheritance. To date, seven genes and two additional loci have been associated with ARCI. Mutations in the seven genes account for disease in 70-75% of the patients.
We performed whole genome homozygosity mapping in a large Iranian ARCI family using high density SNP chips. A large homozygous region of 3.4 Mb on chromosome 5 was observed in all affected individuals. The NIPAL4/Ichthyn gene associated with ARCI lies within the region and mutation screening revealed a homozygous mutation causing p.G297R. Mutation in NIPAL4/Ichthyn has been reported in ARCI patients from Turkey, Syria, Pakistan, Algeria, Colombia and the

Scandinavia countries. Phenotypic similarities and variations among the mutation carrying patients are detected.

P12.115
Molecular analysis of the FLG gene in a sample of Mexican patients with ichthyosis vulgaris

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The Ichthyosis vulgaris (IV) is one of the most common genodermatosis with a semidominant inheritance pattern. IV is characterized by light gray to dark brown scales in extensor areas of extremities and lower abdomen. IV has an incidence of 1:250 to 1:5300 births. FLG gene is located on 1q21, and encodes for the filaggrin protein. Mutations in FLG gene are the cause of IV, being the most frequent reported the R501X and 2282del4 mutations, both in exon 3. The aim of the present study was the molecular analysis of the FLG gene in Mexicans individuals with IV. We included 12 patients with clinical diagnosis of IV and normal activity of the steroid sulfatase (STS) enzyme from the Genodermatosis clínica of the General Hospital of Mexico. All IV patients were analyzed by PCR, RFLPs with NlaIII enzyme and DNA automated sequencing anaysis searching for the R501X and 2282del4 mutations. Based on the proposed clinical scale, 33.3% of patients were diagnosed as mild, 41.6% as moderate and 25% as severe. The molecular study revealed 3 positive patients (25%) for the mutation R501X whereas the rest showed no this type of mutations in the FLG gene. There was no relationship between phenotype and genotype. We found a lower frequency of R501X and 2282del4 mutations in IV in comparison with other populations

P12.116
Unraveling the origin and molecular pathogenesis of *TGM1* c.984+1G>A mutation

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Background. Lamellar ichthyosis (LI) is a nonsyndromic disorder of the cornification caused, in the majority of patients, by transglutaminase-1 (TGM1) deficiency resulting from mutations in both copies of the *TGM1* gene.
Objectives. To study the probably common origin of c.984+1G>A mutation in two apparently unrelated Galician families (NW Spain) carriers of c.984+1G>A at heterozygous and homozygous state. To assess the pathologic effect of the splice-site mutation by bioinformatic tools and splicing assays.
Materials and methods. Microsatellite and SNPs data spanning 12Mb on chromosome 14q11 were evaluated to unravel the common origin in families and 100 control individuals. The effect on splicing was assessed using bioinformatic prediction tools follow by analysis of mRNA from peripheral blood lymphocytes. Molecular modelling was performed to investigate the effect of the variation at the protein level.
Results. Both families shared a common haplotye spanning 0.93Mb no present in control population, confirming the origin from a single mutational event. c.984+1G>A lead to two alternative mRNA splice variants, that included 30 (p.Met329_Val330ins10) and 32 nucleotides (p.Val330MetfsX12) of the 5' of intron 6. This result was in accordance with the presence of two cryptic splice sites predicted by bioinformatic tools. The in frame insertion would create a new loop in the surface of the catalytic domain, which is likely to interfere with the proper folding yielding a structure more susceptible to proteolytic cleavage. Conclusion. Our results suggest that c.984+1G>A is a founder mutation for LI in the Galician population and unravels the molecular pathogenesis of this mutation.

P12.117
Localisation of a gene for rare immuno-osteodysplasia syndrome

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We report high density mapping and sequencing results on a consanguineous family with an immuno-osteodysplasia (IDS). The 3 affected siblings, 2 females and 1 male, presented with a deficient innate immunodeficiency with hypogammagloulinaemia & partial antibody deficiency requiring regular IVIG replacement therapy. In addition, they have a renal tubulopathy leading to potassium and magnesium loss. They have proportionate short stature and abnormalities on X-rays, including epiphyseal delay, flattening of the proximal femoral, distal femoral and proximal tibial epiphyses. The two older children are maintained on oral antibiotic prophylaxia, subcutaneous immunoglobulins & potassium and magnesium supplements. The youngest child, a boy, developed pneumocytis pneumoniae & cryptosporidium positive diarrhoea. He unfortunately died following bone marrow transplantation. Runs of homozygosity (ROH) shared by the 3 affected siblings were identified and subsequently compared to the patterns of homozygosity in 7 unaffected relatives. The three IDS patients shared 450 ROH ranging in size from 7.1 kb to 4.1Mb. A total of 32 ROH, totalling 6.9 Mb and containing 79 genes, were exclusively shared by the IDS patients. The candidate loci were targeted using NimbleGen's sequence capture technology and sequenced in 3 affected and 3 unaffected individuals. We identified 34 homozygous variants (coding regions and utr's) in 18 different genes that segregated with the IDS phenotype. After excluding variants reported in dbSNP, 13 homozygous mutations in 11 different genes remained for further consideration as potential disease-causing mutations. Functional studies may help in elucidating which of the 11 candidate genes is predisposing to IDS.

P12.118
Genetic analysis on twenty eight families with inherited arrhythmogenic disorders: implications for a consanguineous population

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Introduction: Implicated in the etiology of sudden death, inherited arrhythmogenic disorders (IAD) are a group of genetic conditions that are characterized by locus heterogeneity. The majority of IAD is caused by channelopathies and is inherited in autosomal dominant fashion. **Objective**: Molecular characterization of IAD in our highly consanguineous population. **Patients**: 28 Saudi families with various forms of IAD were recruited:13 with Romano-Ward syndrome (RWS), 5 with Jervell and Lange-Nielsen syndrome , 4 with Brugada syndrome, 2 with sick sinus syndrome, 3 with arrhythmogenic right ventricular dysplasia, and 1 with catecholaminegric polymorphic ventricular tachycardia. **Methods**: Direct sequencing for genes (*KCNQ1, KCNE1, KCNH2, KCNE2, SCN5A, GPD1L, DSP, PKP2, DSG2, DSC2, CASQ2*) known to be implicated in IAD and, in consanguineous families with negative gene sequencing, SNP-based genome-wide homozygosity mapping were performed. **Results**: Heterozygous mutations in *KCNQ1* (5 families), and *PKP2* (1 family), and homozygous mutations in *KCNQ1* (4 families), *CASQ2* (1 family), *SCN5A* (1 family) were detected. SNP-based homozygosity mapping identified a potential novel locus on chromosome 5q34 for RWS. Analysis of candidate genes in the region is in progress. **Conclusion**: Our work represents largest cohort of Arab patients with IAD. Our molecular data illustrates the impact of the high degree of consanguinity on the genotypes of IAD; a finding that has an important preventive implication.

P12.119
Mutation Spectrum of MLL2 in a cohort of Kabuki syndrome patients

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Kabuki syndrome is a rare, multiple congenital anomalies/mental retardation syndrome characterized by a peculiar face, short stature, skeletal, visceral and dermatoglyphic abnormalities, cardiac anomalies, and immunological defects. Mutations in the histone methyl transferase MLL2 gene have been identified as its underlying cause. Sanger sequencing of the coding region of MLL2 including intron-exon junctions was performed in 62 index patients. The putative causal and possible functional effect of each nucleotide variant identified was predicted by in silico tools. We identified 46 patients with MLL2 mutations; 42 are novel mutations. The majority are nonsense or frameshift predicted to generate a truncated polypeptide. Yet, we reported 3 indel and 7 missense mutations. Finally 3 MLL2 mutations affected the splicing process resulting in a shorter protein. 26 % (16/62) patients from our cohort with clinical evidences of Kabuki syndrome were negative for mutation in the coding region of MLL2 gene. Mutations in the regulatory regions of the gene, genetic heterogeneity of Kabuki syndrome, and exon micro duplications and/or micro deletions gene might be the possible causes for the lack of MLL2 mutations in such patients. This study emphasizes the relevance of the mutational screening of the MLL2 gene among patients with Kabuki syndrome to improve the actual knowledge on the clinical basis of this syndrome, to design functional studies to understand the molecular mechanisms underlying the disease, to establish genotype-phenotype correlations, to improve the clinical management, and to identify potential targets for therapy.

P12.120
Detection by microarray-CGH of large rearrangements of the LAMA2 gene in 16.8% of MDC1A patients

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The most frequent form of autosomal recessive Congenital Muscular Dystrophies (MDC1A) is caused by mutations of the LAMA2 gene that codes for the laminin alpha-2 protein. This large gene is composed of 65 exons and extends over a genomic region of 633,366 bp. The molecular diagnosis revealed that most mutations are private and therefore the sequencing of all exons is required. Using a series of 113 MDC1A families, we have identified two LAMA2 mutations in 79.7% of families. Therefore, for almost 1/5 of patients, the genetic counseling is difficult as secondary merosin deficiencies can be observed mainly as a result of abnormal O-mannosylation of alpha-dystroglycan as seen in the alpha-dystroglycanopathies associated with mutations in the LARGE, FCMD, POMGnT1, FKRP, POMT1, POMT2 and Fukutin genes.

We therefore developed a new method for detecting and characterizing large rearrangements in the LAMA2 gene based on high-resolution oligonucleotide array-CGH technology. The study of all patients with a clinically diagnosed MDC1A but harbouring only one or no LAMA2 small rearrangement revealed that they indeed harbour one or two large rearrangements of the LAMA2 gene in most cases (16.8% of all MDC1A patients). In parallel, because of the high-resolution of this technique, breakpoints were subsequently characterized by a simple polymerase chain reaction allowing a simple screening test for other family members. This approach, in combination with cDNA sequencing, allowed us to identify LAMA2 mutations in 96.5% of MDC1A patients therefore having a major impact on genetic counseling.

P12.121
Mutation analysis of the LDL receptor gene in Indian families with Familial Hypercholesterolemia

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Background: Familial Hypercholesterolemia (FH) is a metabolic disorder inherited as an autosomal dominant trait characterized by an increased plasma low-density lipoprotein (LDL). The disease is caused by several mutations in the LDL receptor (LDLr) gene. Identification of individuals carrying the defective gene could be useful in reducing the risk of atherosclerosis and myocardial infraction. Our study presents mutation analysis of the LDLr gene in 24 Indian families with FH.

Methods: Genomic DNA was isolated from blood, exon-specific intronic primers were designed and used to amplify DNA samples from individuals.PCR products were directly subjected to automated DNA sequencing to detect the mutations. Alongwith the affected individuals, ten ethnically matched controls were also analyzed to determine the presence of the same mutations.

Result: All the 24 patients had total cholesterol level above 300mg/dl and LDL cholesterol level above 200mg/dl. Sequence analysis of the LDL receptor (LDLr) gene showed 3 novel mutations which have never been reported elsewhere. In exon 10 we reported g.29372_29373insC, which was found in all the 24 patients and was missence mutation coding for C (cysteine) instead of V (valine).

Conclusion: Our study reported 3 novel mutations in 24 Indian families. These novel mutations are predicted to produce change in the amino acid and thus leading to the conformational changes in the structure of LDLr protein. Change in the LDLr protein makes the LDL receptor unable to transport the cholesterol in to the cell and hence cholesterol starts accumulating in the blood stream and leads to FH.

P12.122
Phenotypic characterization of patients with deletions in the 3'-flanking SHOX region.

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The *SHOX* gene is located on the short arm of the X-chromosome in the pseudoautosomal (PAR) 1 region and escapes X-inactivation thus showing a pseudoautosomal pattern of inheritance. Leri-Weill dyschondrosteosis is caused by haploinsufficiency of the *SHOX* gene in 60-70%, and in 15% by deletions of a putative enhancer sequence in the 3'-flanking region. The precise localization of the enhancer sequence is unknown.

We collected clinical data from patients and their relatives with different deletions in the 3'-PAR region, detected by the MRC-Holland MLPA kit (P018-D). PAR probes were numbered 1-12 where nr 1 is SALSA probe 5642-L05096.

Twelve individuals carried a large deletion starting in PAR1 and extending to PAR8/9 or to the flanking CSF2RA probe while 4 individuals had a PAR3-CSF2RA deletion. Thirty-two individuals carried a smaller deletion of 3 probes (PAR4-6), just 5' to the previously described common deletion interval.

Median height SDS, sitting height/height ratio SDS and the presence of Madelung deformity in patients with PAR4-6 deletions were -1.8, +1.4, and 67%, in comparison to -2.4, +1.9 and 42% in patients with larger deletions. The index patients had a median height SDS of -2.7, shorter than their affected parents (-2.0), but disproportion and the presence of Madelung deformity were similar. However, variability of the phenotype in the whole group of patients was remarkable.

We conclude that the critical interval of the enhancer region may be larger than previously suggested.

P12.123
Mutation Analysis of Limb Girdle Muscular Dystrophies in the Czech Republic

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Limb girdle muscular dystrophies (LGMDs) are a group of disorders characterised by atrophy and weakness of proximal limb girdle muscles.

Limb girdle muscular dystrophy type 2A (LGMD2A) is an autosomal recessive disorder caused by mutations in the *CAPN3* gene (15q15) that encodes the muscle specific protein, calpain-3 (p94). LGMD2A is the most frequent form of LGMDs in many European countries.

Another relatively common forms of LGMD are LGMD2I and LGMD2D. LGMD2I is caused by mutations in the *FKRP* gene (19q13.3) that encodes a protein which participates in the glycosylation of α-dystroglycan in the muscle fibre. LGMD2D is caused by mutations in the α-sarcoglycan gene (*SGCA*). *SGCA* (17q21) is an a integral membrane glycoprotein that forms part of the dystrophin associated glycoprotein complex.

We performed analysis of the *CAPN3*, *FKRP,* and *SGCA* genes in a cohort of patients with preliminary diagnoses of LGMD at both the mRNA level using reverse transcription-PCR or at the DNA level using PCR and direct sequencing.

We screened 203 unrelated patients. 50 patients were found to carry *CAPN3* defects. The homozygous occurence of the most common mutation in the *FKRP* gene (p.Leu276Ile) was found in 8 patients. Mutations in the *SGCA* gene was detected in 4 patients. In total, we determined mutations in 62 Czech LGMD patients (31 %).

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P12.124
A novel CAPN3 gene mutation in a LGMD patient from southern Italy

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Limb girdle muscular dystrophies ($LGMD_S$) is a heterogeneous group of inherited progressive muscle disorders affecting predominantly the shoulder and pelvic girdle muscles. There are at least 19 different subtypes of LGMD, 7 with autosomal dominant and 12 with autosomal recessive pattern of inheritance. Immunostaining performed on muscle biopsy and genetic analysis can discriminate between the LGMD subtypes.

LGMD2A is the most prevalent form of recessive LGMDs since it generally accounts for 30% of LGMD2 in Caucasian populations. In a LGMD patient from southern Italy we identified a novel mutation (Gln142Arg) in the exon 3 of the CAPN3 gene, in heterozygosis with the A236T variant previously described by others as polymorphism. We already reported another LGMD patient with the same A236T variant in heterozygosis with a null mutation (R748X) in the exon 21 of the CAPN3 gene. Both patients showed elevated serum Creatine Kinase level, late onset myopathy with subjective weakness of the lower limbs. We also found the A236T variant in 7 unrelated LGMD2 patients from the same geographic area.

These findings suggest that the combination of A236T polymorphism in heterozygosis with a mutation in the CAPN3 gene might be responsible for mild LGMD2A phenotypes. However, demonstration of the pathogenic nature of this variant required the examination of the transcription product.

P12.125
Clinical genetic analysis of LGMD2I in Russia.

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The limb-girdle muscular dystrophy type 2I (LGMD2I) is caused by mutations in the FKRP gene. Being one of the most common forms of the autosomal recessive LGMDs in Europe, its prevalence, the mutation spectrum and symptoms of LGMD2I in Russia are unknown.

We characterized the frequency of LGMD2I in a cohort of 74 Rusian muscular dystrophy patients using DNA sequence analysis. FKRP mutations were found in 9,5% (7/74). Pathogenic changes in both alleles of the FKRP gene were found in 5 patients. In two cases, changes were found only in one allele. Of five founded mutations three were first described (missense: c.265C>T, c.1078G>C, stop codon: c.229C>T). The c.826C>A change, presented in four patients, was the most prevalent mutation.

All patients were women aged 4 to 52. An age of onset varied greatly (from 3 to 32 years). Correlations between the age of onset and the clinical presentation severity weren't found. Clinically, LGMD2I patients exhibit a great phenotypic variability ranging from a severe, rapidly progressive weakness and wasting of the limb-girdle muscles to mild disorders, but all clinical symptoms were typical for LGMDs. Of seven patients only one girl of 9 years old has been wheelchair-bounded since she was seven years old. None of them still has the cardiomyopathy. Three patients had a calf pseudohypertrophy. Contractures of large joints were found in two patients having manifestation in their childhood. The creatine kinase activity varied from 1590 to 14197IU and hadn't correlations between ones and a severity of clinical presentation.

P12.126
Investigation of *ECM1*(1,2,3,4,5,9 and 10 exons) mutation in lipoid proteinosis (*LP*) and genotype-phenotype correlation in Iranian patients

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Lipoid Proteinosis is an autosomal recessive disorder that results from mutations in extra cellular matrix protein(*ECM1*). The clinical signs of *LP* are hoarseness, skin lesions, beaded eyelid papules. During childhood, the skin may be easily damaged by minor trauma or fraction, resulting in blisters and scar formation in Pox-like or cuneiform scars are particularly visible on the face to hair loss. The mucosae of the pharynx, tongue, soft palate, tonsils and lips are also infiltrated and this may lead to respiratory difficulty, sometimes requiring tracheotomy.

We aimed to screen 10 Iranian families clinically diagnosis with *LP*. Genomic *DNA* was extracted from all the patients and the parents. *PCR* was employed to amplify all 10 exons of *ECM1* gene. The *PCR* products were then sequenced using *ABI* sequencer.In this report, we are going to present our data regarding *ECM1* gene mutations in Iranian *LP* patients. To our best knowledge this is the first reported molecular data of Iranian *LP* patients

P12.127
Exclusion of known lymphedema genes in two families with severe congenital lymphedema.

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Lymphedema is a soft tissue swelling resulting from abnormal accumulation of interstitial fluid
containing high molecular weight proteins due to abnormal drainage of lymph by the lymphatic vasculature. Primary lymphedema is due to an abnormal lymphangiogenesis which usually starts *in utero*. Some of the cases are inherited and in most cases have autosomal dominant or recessive mode of inheritance. Some of the autosomal dominant cases have incomplete penetrance and variable expression. Both syndromic and non-syndromic cases have been caused by mutations in genes with major role in lymphangiogenesis including FOXC2, VEGFR3, SOX18, CCBE1, PTPN14 and GCJ2.

We report two consanguineous families with recurrence of an autosomal recessive form of congenital lymphedema. One of the families is of Iranian-Jewish and Israeli descent and the other of Iraqi descent. Direct sequencing of the known lymphedema genes including coding sequences and intron-exon boundaries, did not identify any mutation. Whole genome scan using Affymetrix SNP-Chip 250K was thus performed in both families and no copy number change was identified in the affected members. Autozygosity mapping and parametric linkage analysis also excluded the candidate lymphedema-genes. Three overlapping autozygous regions were identified in the two families and were confirmed by linkage analysis.
Our findings provides evidence for the existence of new causative gene or genes associated with the autosomal recessive form of congenital lymphedema. (E-mail : Miikka.vikkula@uclouvain.be).

P12.128
Use of exome sequencing to identify GJC2; a gene responsible for causing autosomal dominant primary lymphoedema

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Genotyping array analysis was carried out on two families, all affected by four-limb, primary lymphoedema and linkage to chromosome 1 (LOD 3.54) was identified. The region was approximately 17Mb (150 genes). Instead of analysing these genes using traditional Sanger sequencing, Next Generation Sequencing was employed. Exome sequencing of a proband from one of the families was performed using the Agilent human All Exon Capture Assay and sequenced on the Illumina GAIIx platform with 76bp paired end reads. The sequence reads generated were aligned to the reference human genome sequence (hg18) and the location of variant substitutions and small insertion/deletions defined. Analysis of the region of the genome previously shown to cosegregate with the disease status (chromosome 1 delimited by rs10494988 to rs1043909) revealed only two novel nonsynonymous variants in *NVL* (V746M) and in *GJC2* (S48L). Both changes cosegregated with the affected status in the two families and subsequent sequencing of these genes in 19 unrelated individuals with four-limb lymphoedema revealed another two patients with the S48L change. Two additional nonsynonymous *GJC2* variants in two patients were identified, one unreported (M210R) and one published (P316L). Recently it has been suggested that *GJC2* is a pathogenic gene in lymphoedema (Ferrell et al 2010). Our report describes a novel variant in *GJC2* and confirms the S48L and P316L changes described by Ferrell et al (2010). We have therefore been successful in demonstrating how exome sequencing of one patient, together with linkage analysis, enabled rapid identification of a new gene for primary lymphoedema.

P12.129
Insertion of an SVA element, a non-autonomous retrotransposon, in *PMS2* intron 7 causes Lynch syndrome

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Germ line mutations in the mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2* cause Lynch syndrome, an autosomal dominant inherited form of cancer predisposition. With an RNA-based mutation detection protocol we found a 71 bp insertion between *PMS2* exon 7 and 8 in the mRNA of one suspected LS case. With Southern blot analysis a heterozygous 2 kb insertion in the genomic DNA of the patient had been found before but the insert could not be amplified at that time. A BLAST search of GenBank with the inserted 71 bp mRNA sequence returned a match with high homology against part of an SVA repeat on chromosome 7q11.23. We now report that the insert is a 2 kb long 5'-truncated SVA element consisting of a VNTR region of 1.4 kb, a SINE-R region and a polyA tail of $(A)_{66}$. This is the first report of an SVA insertion as cause of Lynch syndrome. The inclusion in the mRNA of the first 57 nucleotides of the SVA element together with 14 intronic nucleotides upstream causes a premature termination codon that triggers nonsense mediated RNA decay of the aberrant *PMS2* transcript. The SVA insert is not detectable by current diagnostic testing methods such as MLPA and direct Sanger sequencing on genomic DNA. Probably such a mutation will also be missed with next generation sequencing approaches.

P12.130
MtDNA dysfunction in a transgenic mouse model of Machado-Joseph Disease(MJD)

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Background: We investigated the potential of mtDNA dysfunction as a biomarker of disease progression in MJD using a novel transgenic (TG) mouse model, expressing full-length human ataxin-3 and displaying a motor phenotype (strain FVB/N, line CMVMJD94).
Methods: DNA was obtained from affected (pontine nuclei - Pn) and non-affected tissues (hippocampus - Hp and blood - Bl) of TG mice. Three distinct age-groups were considered: 8, 16 and 24 weeks (before onset, at onset, and at well established phenotype, respectively). Wild-type mice (wt), serving as controls, were analyzed for the same tissues and age groups. Quantitative changes (changes in copy number) as well as qualitative changes (ΔmtDNA3867 deletion) in mtDNA were studied by Fluorescent based quantitative PCR (FQ-PCR) in a total of 177 samples (84 TG and 93 wild-type). The absolute quantities of mtDNA/nDNA were compared by the "absolute quantitation" method.
Results: A clear pattern of mtDNA copies decrease with age was observed for both wt and TG mice (1.06-2.53-fold and 1.24-2.58-fold, respectively). Affected tissue showed higher level of mtDNA copies decrease (1.21 times higher in TG mice) and higher level mtDNA deletions (6.05% more in TG mice). The highest amount of mtDNA deletions was observed in 8 weeks old mice, with higher values for TG mice (49.75-72.75% vs. 47.60-58% of wt animals), decreasing with age.
Conclusions: Our study confirms an association between mtDNA depletion with progression of phenotype in TG mice and indicates that the mtDNA 3,867 bp deletion may play a role in the early stage of the disease.

P12.131
Characterization of prelamin A isoforms accumulated in Mandibuloacral Dysplasia type A (MADA) cells

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Mandibuloacral dysplasia type A (MADA) is a rare autosomal recessive disorder characterized by craniofacial defects, skeletal manifestations, cutaneous changes and partial lipodystrophy. MADA is caused by mutations in the LMNA gene encoding lamin A/C. The molecular features consist in the accumulation of the unprocessed lamin A precursor (prelamin A) at the nuclear rim and in nucleoplasm, with a linear increase of protein amount in older patients. The accumulation of 74 kDa protein, containing a histidine residue in position 527 in C-terminal domain, is caused by wrong process of post-translational modifications and cleavages with slowed down formation of intermediate forms and final mature lamin A.
To find out the prelamin A forms recovered in MADA cells, we performed the immunofluorescence analysis using three different antibodies detecting all forms of prelamin A, both non-farnesylated and farnesylated full-length prelamin A, and an intermediate product carboxymethylated prelamin A.
We show that full-length prelamin A either farnesylated or non-

farnesylated is detectable in middle and high passage fibroblasts, but it's not observed in cells at low passages. Carboxymethylated prelamin A is slightly expressed and detectable only in middle passages.
In conclusion, we show that protein processing changes depending on the passage numbers and the age of patients, suggesting the onset of a feedback mechanism.

P12.132
No evidence for involvement of excess free transforming growth factor-β in disturbed signal transduction in Marfan syndrome

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Background: Marfan syndrome is an inherited connective tissue disorder which is usually caused by mutations in the fibrillin-1(FBN1) gene. In the ethiology, transforming growth factor β(TGF-β) plays an important role. Inactive TGF-β is stored in the large latent TGF-β complex (LLC) in the extracellular matrix, bound to fibrillin-1. Mutations in the FBN1 gene are supposed to impair binding of LLC and cause increased free TGF-β and increased signalling. Activated TGF-β-receptors phosphorylate Smad3, to pSmad3, which in turn is transferred into the nucleus by Smad4, where it is involved in regulation of genes involved in growth, differentiation, inflammation and apoptosis.
Methods: We studied these proteins by immunohistochemical staining of paraffin embedded aortic tissue from 5 Marfan patients, all with a pathogenic FBN1 mutation, that underwent surgery for thoracic aortic aneurysms. Control tissue (n=5) was obtained at autopsy of individuals without aortic aneurysm. Quantification of immuno-staining was performed using Mirax-Scan(Zeiss) and 3D-Histech Pannoramic Viewer1.14.
Results: In controls and patients, TGF-β showed extensive staining in the tunica adventitia. In tunica media no TGF-β was detectable, except in areas adjacent to rupture. Intact areas of the tunica media showed no staining of TGF-β, but did show significantly increased staining of total Smad3 (p=0.006), Smad4 (p=0.001) and nuclear staining of pSmad3 (p=0.048), in patients compared to controls,
Conclusion: We conclude that TGF-β signal transduction is disturbed in Marfan patients with a mutation in FBN1, but the underlying mechanism is not clear. There is no evidence for involvement of excess free TGF-β.

P12.133
MLPA analysis of *FBN1* and *TGFBR2* gene in Marfan syndrome and associated disease: results of a large French study

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Marfan syndrome (MFS) is a prevalent connective tissue disorder with manifestations in the skeletal, ocular, and cardiovascular systems. In most cases MFS is caused by heterozygous mutations in the gene encoding the extracellular matrix protein fibrillin-1 (*FBN1*). Heterozygous mutations in the genes coding for transforming growth factor beta receptors I (*TGFBR1*) and II (*TGFBR2*) have also been reported in patients with MFS2 and MFS-related disorders.
In classical MFS, mutation analyses fail to detect *FBN1* involvement in about 10% of cases, possibly suggesting the limitations of commonly used standard PCR-based screening approaches.
In this study, we searched for partial or complete deletions/duplications of *FBN1* and *TGFBR2* genes using MLPA technique in a cohort of 206 patients with suspected MFS or MFS-like phenotypes, negative for point mutations in the *FBN1, TGFBR1* and *TGFBR2* genes.
16 abnormal patterns were found representing 14 deletions and 1 duplication in *FBN1*, and 1 deletion in *TGFBR2*. Real-time quantitative PCR confirmed 15 abnormal patterns. In one case, the abnormal pattern was due to an insertion of an interspersed *Alu* element in the target sequence of the MLPA probe.
Taken together, the frequency of *FBN1 or TGFBR2* deletions or duplications in patients with suspected MFS without point mutation in *FBN1*, *TGFBR1*, and *TGFBR2* can be estimated as 15/206 (7%). *FBN1* and *TGFBR2* gross deletions or duplications could thus be responsible for 0.5 to 1% of MFS cases. However, the relatively high cost of the MLPA technique raises the question of its routine use in a diagnostic laboratory.

P12.134
Marfan syndrome with a complex chromosomal rearrangement including deletion of the *FBN1* gene

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Mutations in the *FBN1* gene, mapped at 15q21.1, are the major cause of Marfan syndrome (MFS), an autosomal dominant connective tissue disorder, which displays variable manifestations in the cardiovascular, ocular, and skeletal systems. Only four cases with deletions of the whole *FBN1* gene detected by molecular cytogenetic techniques were found in literature. In only two of these patients, there was the presence of clinical features of the Marfan syndrome spectrum associated to mental retardation. Chromosomal alterations in Marfan syndrome, such as interstitial deletions involving the 15q21.1 band are rare. There is no described patient with MFS and complex chromosome rearrangement (CCR) involving more than two breakpoints on two or more chromosomes. We report a 16-year-old female with MFS spectrum (positive thumb and wrist sign, scoliosis, joint hyperlaxity, high-arched palate with dental crowding, dysmorphism, mitral insufficiency with dystrophic valve, striae) associated to intellectual deficiency. Due to atypical marfanoid phenotype, cytogenetic study was performed using G-banding that revealed a *de novo* balanced translocation involving chromosomes 6, 12 and 15 with breakpoints apparently at 6q22, 12q24 and 15q21. Whole genomic array using Cytogenetics Whole-Genome 2.7M Array (Affymetrix) showed a 1.9 Mb deletion in the 15q21.1 region, including the *FBN1* gene. Fluorescence in situ hybridization (FISH) with painting and bacterial artificial chromosome probes revealed a much more complex rearrangement with eight breakpoints as follows:
46,XX,t(6;12;15)(6pter→6q13::15q15.1→15q21.1::15q21.1→15q22.1::6q14.1→6q16.3::12q24.22→12qter;12pter→12q24.21::6q21→6q22.2::15q22.2→15qter;15pter→15q14::6q22.31→6qter).arr 15q21.1(45,466,733-47,335,104)x1. This is the first report of MFS with a complex chromosome rearrangement with a deletion of the *FBN1* gene and contiguous genes. (Financial support: FAPESP, Brazil).

P12.135
49 Novel mutations of Marfan syndrome in Czech population

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Marfan syndrome (MFS) is a heritable autosomal dominant disorder of connective tissue with prevalence of between 1 in 5-10 000. Approximately 25% of MFS patients are sporadic cases due to new mutations. MFS is noteworthy for its clinical variability. Major features of the MFS include cardiovascular disorders (dilatation and dissection of ascending aorta), eye disorders - *ectopia lentis*, defects of skeletal system - *pectus carinatum*, *pectus axcavatum* and/or other diagnostics criteria as *arachnodactyly*.
MFS is caused by mutations in fibrillin 1 gene (*FBN1*) resulting in defective glycoprotein fibrillin-1. *FBN1* is located on chromosome 15 at locus q15-q21.1. Recently, there are showed that three other genes *FBN2* (5q23-q31), *TGFBR2* (3p22) and *TGFBR1* (9q22) influence MFS. Mutations in these genes and their phenotype manifestations are also in Loyes-Dietz syndrome, Marfan syndrome type II. and thoracic aortic aneurysm.
The molecular analysis includes mlpa (multiplex ligation-dependent probe amplification), separation of PCR products by SSCP (single-strand conformation polymorphism) and sequencing.
We have performed mutation detection on 395 patients with suspected MFS. There were detected 49 novel mutations.

P12.136
Complex mutation screening of FBN1 and TGFBR2 genes in patients with Marfan and marfan-like syndromes by different highly sensitive methods (DCODE Universal Mutation Detection System, HRM, Sequencing)

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Marfan syndrome (MFS) is an inherited autosomal dominant connective tissue disorder. Abnormalities appear in skeletal, ocular and cardiovascular systems. The main cause of MFS are mutations in the fibrillin1 gene (FBN1). Recently, the transforming growth factor beta receptor 2 gene (TGFBR2) has been shown to be associated with a second type of this disorder with typically mild or absent ocular involvement (MFS type 2) as well as with classical MFS. Currently we analyzed some regions of FBN1 and TGFBR2 genes in 80 patients with MS and marfan-like syndromes from different areas of Russia by several methods: SSCP, TTGE, Heteroduplex Analysis on the BioRad DCODE Universal Mutation Detection System and HRM-method on Bio-Rad CFX96 system; and sequencing on MegaBace 1000. We identified two missense mutations (G1176Y in 28 exon and C2489Y in 60 exon) which affects cbEGF-like motifs of fibrillin-1 protein in two patients with classical MFS symptoms. We also found 9 polymorphisms both in coding and non coding regions of FBN1 gene, five of them are not previously described. One novel mutation (670C>T; T223M) has been found in TGFBR2 gene in two unrelated patients with marfan-like syndrome who did not fulfill Ghent nosology and who did not have mutations in FBN1 gene. Mutation T223M affects highly conserved serine/threonine protein kinases catalytic domain that leads to change phospho transferring status of TGFBR2 protein. In addition two novel polymorphisms have been found in intronic regions of TGFBR2 gene. Mutation screening of FBN1 and TGFBR2 genes will be continuing.

P12.137
Genetic analysis of Marfan syndrome

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The Marfan syndrome is a genetic disorder of the connective tissue which has many different clinical features most of them affecting the skeleton, skin, and joints. The varieties are known as type I (dominant autosomic disease related to FBN1 gene) and type II associated to gene TGFBR2. Whereas FBN1 gene codify for a protein known as fibrillin-1 which is needed for the formation of elastic fibers of connective tissue. However, TGFBR2 gene codify for the receptor 2 of a growth factor. The aim of this poster is to present the analysis done in these both genes in eight patients from an Andalusian population (South of Spain), diagnosed of Marfan syndrome. The extraction of DNA was done by an organic procedure with phenol/chlorophorm and the amplification method was using VariantSEQr® (Applied Biosystems). The sequencing process was done in adjacent codifying and intronic regions of both genes.

As a result, we could observe four Nonsense mutations in four to eight patients. Two of them (p.R2220X and p.R1125X), have been previously described in international data bases (HGMD, Uniprot) in Marfan syndrome type I. However, the others (p.E1133X and p.Q1419X) have never been cited in any data base. It has also described five Missense mutations in four to eight patients and similar results from the above described has been noted. Two of them are cited in data base (p.R122C and p.G343R) for causing Marfan syndrome type I, whereas the others (p.C830G, p.S1345I and p.P1258S) are the first time to be notice in this syndrome.

P12.138
Novel, recurrent mutations and a complex rearrangement in the MECP2 gene

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Rett syndrome (RTT) is caused by mutations in the methyl-CpG-binding protein 2 (MECP2) gene. The MECP2 gene has some unique characteristics: 1) it is mainly affected by de novo mutations, due to recurrent independent mutational events in a defined "hot spot" regions or positions; 2) complex mutational events along a single allele are frequently found in this gene; 3) most mutations arise on paternal X chromosome. The recurrent point mutations involve mainly CpG dinucleotides, where C>T transitions are explained by methylation-mediated deamination. The complex mutational events might be explained by the genomic architecture of the region involving the MECP2 gene. The finding that most spontaneous mutations arise on paternal X-chromosome supports the higher contribution of replication-mediated mechanism of mutagenesis. We present 9 types of mutations in the MECP2 gene, detected in a group of 22 Bulgarian and 6 Romanian classical RTT patients. Thirteen patients were clarified on molecular level (46.4%). The point mutations in our sample account for 61.5%. One intraexonic deletion was detected in the present study (7.7%). A novel insertion c.321_322insGAAG, p.(Lys107_Leu108insGluAlafs2*) was found (7.7%). Large deletions and complex mutations account for 23%. A novel complex mutational event c.[584_624del41insTT; 638delTinsCA] was detected in a Romanian patient. Complex gene rearrangements involving a combination of deletions and insertions have always been most difficult to detect, to specify precisely and hence to explain in terms of their underlying mutational mechanists.

P12.139
Exome sequencing in siblings with intellectual disability

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The extensive clinical and genetic heterogeneity of Intellectual Disability (ID) poses a major challenge for the identification of the underlying molecular causes both in research and diagnostic laboratories. Recent and fast technical developments allow to sequence the complete coding sequence of a human genome of one individual at once (exome sequencing). This has revolutionized clinical genetic research, offering new opportunities to identify the genetic cause in ID patients and is expected to become implemented soon in the clinical practice as a diagnostic tool.

We have collected families with at least two individuals with (non)-syndromic ID that are suggestive for an autosomal recessive inheritance pattern. In these families, we perform exome sequencing on genomic DNA of one individual of each family. Stringent filtering with an in-house developed pipeline allows fast identification of potential homozygous and compound heterozygous mutations. In addition, homozygosity mapping with a 250K SNP genotyping array has been performed to identify overlapping homozygous genomic regions in siblings because we expect a significant contribution of pathogenetic homozygous mutations. So far, exome sequencing has been performed for 15 families and potential mutations are being validated. Next, we aim to identify additional mutations in our candidate ID genes in a cohort of patients with a comparable phenotype to confirm involvement of the respective gene in ID.

This study will contribute to identification of genes that cause recessive ID and will help to implement exome sequencing for autosomal recessive ID into the diagnostic setting.

P12.140**

A first systematic evaluation of Intellectual Disability gene function in neuronal development

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More than 440 genes have been implicated in Intellectual disability (ID) to date. They provide an exciting molecular window into the neurobiology of cognition and brain development. However, little is known about the role of most ID genes in the nervous system. To change dimensions of research in this field, we use *Drosophila* to systematically uncover ID gene function.

In order to reveal ID gene function in flies, large scale RNAi screens are performed in which relevant aspects of neuronal architecture, functioning and behavior are quantitatively assessed. Because genes that cause similar phenotypes likely operate together, our functional data are further subjected to bioinformatics analysis to predict groups of ID genes that act in the same pathways. We aim to dissect these pathways and identify novel molecular players and interactions.

In my PhD project, I have systematically dissected the role of all fly orthologues of human ID genes in neuronal function and development. My RNAi screen was focused on *Drosophila* photoreceptor neurons, for which I have established a phototaxis assay. This assay is used as a simple read out to measure photoreceptor functionality.

I have recently completed the primary RNAi screen, which yielded many interesting results. Ablation of ID genes resulted in morphological phenotypes in 40% of all cases and 9% gave functional/phototaxis phenotypes. Strikingly, many of these first hits appear to be related to microtubules, actin cytoskeleton, mitochondria or membrane/vesicle trafficking.

I will present these findings and my follow up approach using secondary assays such as histology and electrophysiology.

P12.141

Identification of MAN1B1 as a gene with elevated mutation frequency in non-syndromic autosomal recessive intellectual disability

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We have recently identified an overlapping homozygosity-by-descent locus on chromosome 9q34.3 (MRT15) by genome-wide genotyping in 4 consanguineous families with non-syndromic autosomal recessive ID (NS-ARID) from Pakistan, and 1 from Iran. Enrichment of the exonic regions within the respective linkage interval followed by next generation sequencing revealed a missense mutation (R334C) in the MAN1B1 gene, segregating with ID in the Iranian family. Mutation screening in the Pakistani families revealed a homozygous nonsense mutation (W473X) affecting MAN1B1 in one family, and an additional missense mutation (E397K) in this gene segregated in 3 families from the same village with likely shared inheritance. Both missense mutations affect amino acid residues that are conserved throughout the animal kingdom and potentially disrupt the oligosaccharide binding properties at the enzyme's catalytic domain. MAN1B1 encodes the mannosyl oligosaccharide alpha 1,2-mannosidase, which belongs to the glycosyl hydrolase family 47 (GH47). GH47 proteins are believed to be key enzymes in the maturation of N-glycans in the secretory pathway and seem to be involved in the timing and disposal of misfolded glycoproteins through the endoplasmic reticulum-associated degradation (ERAD) pathway. Unlike patients suffering from the lysosomal storage disease, mannosidosis (caused by mutations in lysosomal α-mannosidase enzyme MAN2B1, and lysosomal β-mannosidase enzyme MANBA) who present with a variety of syndromic features, the patients from all 5 families identified with MAN1B1 suffer from purely non-syndromic ID. Thus we presume that here a different pathological mechanism, e.g. disruption of the ERAD pathway, is involved.

P12.142

1qter microdeletion syndrome: molecular and clinical analysis of 8 patients and characterization of a critical region for mental retardation

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The 1qter microdeletion syndrome with microscopically visible deletion of the distal part of the long arm of chromosome 1 (1q42, 1q43 and 1q44) has a well known phenotype which was characterized by Johnson *et al.* in 1985. It includes mental retardation (MR), severe progressive microcephaly, growth retardation, seizures, variable cerebral, cardiac, urogenital, gastro-oesophageal abnormalities and dysmorphic facial features (round face, epicanthal folds, upslanting palpebral fissures, short broad nose, smooth philtrum, downturned corners of the mouth and thin vermilion border of the lips, microretrognathia, palatal defects and low set ears).

Several studies have focussed on cerebral anomalies and have defined critical regions for MR, microcephaly and corpus callosum anomalies, however differing in size and location. Recently, in a series of 4 patients, Caliebe *et al.*, 2010 pointed out the *HRNPU* gene (1q44) as potentially involved in corpus callosum anomalies.

We report the characterization of the deletion in 8 patients with pure 1qter microdeletion syndrome by array-CGH (44K Agilent chip) and analysis of their phenotype. By pooling clinical and molecular data of our 8 patients with those of 65 patients from the literature, we were able to define in this largest series of 1qter microdeletion patients, a 450kb critical region for MR.

This region includes 3 genes *FAM36A*, *HNRPU* et *EFCAB2* which are candidates for brain development.

P12.143

Non-syndromic XLMR due to a novel frameshift mutation in *MED12*

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X-linked mental retardation (XLMR) is a common disease arising from many mutations along the X chromosome. They have been divided into syndromic and non-syndromic according to the association of

additional features. Identification of genes causing non syndromic XLMR has long been challenging but large-scale systematic resequencing has been shown to be promising for the discovery of rare, disease causing sequence variants.
We report on a family including 10 affected males with severe MR and several females with variable cognitive impairment consistent with X-linked recessive inheritance. Male patients all had severe mental retardation and behavior problems. Dysmorphic features included long narrow face, malar hypoplasia, large forehead, full nasal root, thin nose, small philtrum. Head circumference was normal. They had no marfanoid habitus or visceral malformations. Brain MRI, in two patients, showed isolated moderate cerebral atrophy. Most affected females were moderately mentally affected but one of them had severe MR with autistic features.
The initial linkage study revealed a localization region from Xp11.21 (AlaS2) to Xq22.3 (COL4A5) in 6 of 7 affected males. A novel, unreported frameshift mutation was identified in the *MED12* gene by exome sequencing performed within the EuroMRX consortium. The mutation segregated with disease in all affected males. *MED12* encodes a subunit of the mediator complex, which serves as an interface between transcription factors and RNA polymerase II. Two recurrent missense mutations in *MED12* have been previously reported in Lujan-Fryns syndrome and in FG syndrome type 1 (Opitz-Kaveggia). The present data extends the phenotypic spectrum associated with *MED12* mutations.

P12.144

Search for mutations and pathogenic haplotypes in hemizygous genomic regions of patients with intellectual disability (ID)

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Background: Molecular Karyotyping in intellectually disabled children has revealed family-specific deletions which do not represent known CNVs. Such deletions could either be harmless familial variants or recessive mutations. If the latter is true, the affected child should carry an additional mutation on the homologous chromosome. Since autosomal recessive inheritance is supposed to account for nearly a quarter of all individuals with non-syndromic MR, the likelihood of finding a second mutation in children with an inherited deletion is substantial.
Experimental approach: Sanger-sequencing of coding exons of selected genes. Criteria for gene selection: complete or partial deletion of one allele plus expression in brain. Selected genes: *SYT11, NRXN1, ARPP-21, HIST1H2AG, HIST1H2AH, HIST1H2BJ, ANLN, EXOC4, LINGO2, VCL, ALDOA, DOC2A, ASPHD1, KCTD13, RAB37,* and *SULT4A1.*
Results: Besides several SNPs in various candidate genes we have identified three unclassified variants in *LINGO2* (p.Asp135Asp, p.Arg369Gln, p.Asn522Tyr) in three unrelated children with ID. Since all of these children have a partial *LINGO2*-deletion as well, the observed variants may represent recessive mutations.
Future work: Since LINGO1 was shown to be critical for CNS myelination in mice, the closely related LINGO2 may also be critical for mammalian brain development. As a first step to elucidate a clinical relevance of our *LINGO2* variations, we are going to quantify *LINGO2* expression on cDNA level in our ID-patients with a *LINGO2* deletion, as well as in the corresponding healthy family members. Furthermore, we will determine the allele frequency of the *LINGO2* variants mentioned above by genotyping 500 healthy individuals at these loci.

P12.145**

Pitfalls in the diagnosis of X-linked mental retardation: next generation sequencing reiterates the role of *ATRX* in both syndromal and non-syndromal XLMR

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We report the 15 year evolution of genetic analysis in a non-consanguineous Ashkenazi-Jewish family. Three male sibs were affected with mild-moderate MR, variable dysmorphism, and one also with microcytic-anemia partially responsive to iron replacement. Karyotypes, FraX testing were normal. Marker analysis demonstrated linkage to 47Mb X-pericentromeric region (DXS990-DXS1003; LOD score 1.8), two female sibs were predicted carriers, one bore an affected son. X-inactivation didn't reveal skewing in obligate/predicted carriers. Sequencing of *RSK2, ACSL4, ARX, FTSJ1, GDT1, IL1RAL1, JARID1C, OPHN1, PQBP1, ZNF41* and *ATRX* (helicase& zinc-finger domains), and array-CGH were normal. An Xq23 1.5Mb duplication was identified in affected and unaffected males, therefore regarded unrelated to MR. One affected male was included in large-scale Sanger-sequencing X-exon study (718 genes, Tarpey, Nature Genetics 2009); no mutation identified.
We performed unbiased capture& sequencing of all X-exons (Agilent SureSelect X-Exome kit, Illumina GAIIx platform), and identified a mutation in 5' region of *ATRX:* c.109C>T, p.R37X. This mutation has been reported by Guerrini (Neurology, 2000) in a family with mild MR and epilepsy, and subsequently identified in original Chudley-Lowry family (Abidi, European Journal Human Genetics, 2005). The mild phenotype of this nonsense mutation is related to downstream alternative-translation initiation. In contrast to other cases, this family didn't have skewed X-inactivation or epilepsy. In retrospect, it became clear that Tarpey's study did not include *ATRX* 5' exons. We have subsequently identified two other families with ATRX-R37X, and conclude that this could be an overlooked cause of mild XLMR, which may not be covered in XLMR testing-panels.

P12.146

Identification of novel X-Linked Mental Retardation mutations in Israeli population

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Mental retardation affects 1%-3% of the general population and is characterized by malfunctioning of the central nervous system, resulting in significant intellectual disability. Genetic etiologies are found in about half of the cases and include chromosomal or structural abnormalities, genomic disorders and single gene diseases. A significant portion of known mental retardation disease-causing genes resides on human chromosome X, whereby the affliction is more prevalent in males. Despite extensive investigations, the molecular basis for many X linked mental retardation (XLMR) cases has not been reveled by classical genetic approaches, leaving numerous families without prenatal diagnosis. Next-Generation-Sequencing techniques open new avenues in the elucidation of such Mendelian disorders. We have recruited 6 Israeli families of different ethnic origins, with a total of 21 affected males, showing both syndromic and non-syndromic XLMR. Among syndromic families, XLMR is accompanied with neurological anomalies causing a neurodegenerative disease and early death. To uncover the disease causing mutations, we design a novel X chromosome 'super-exome' capture kit, which includes all coding and non-coding exons, with extended splice junctions, RNA genes, as well as regulatory and highly conserved genomic regions. In parallel, we are developing next-generation-sequencing bioinformatics tools suitable for super-exome analyses. Deciphering the disease-causing mutations will shed new light on the mechanisms of mental retardation with implications to other neuronal deficits, and would provide such families with accurate genetic testing and prenatal diagnosis.

P12.147
Deletion or disruption of *CNKSR2*, a scaffold protein in the RAS/MAPK-pathway, causes X-linked mental retardation

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In our diagnostic laboratory aberrations of *CNKSR2* were discovered in two boys with developmental delay: The first was a 5-years old boy with microcephaly and epilepsy. He had a 234 kb deletion removing 17 of 21 *CNKSR2* exons. The deletion was inherited from his healthy mother. Further family follow-up is ongoing. The second patient is a 12-years old boy with a *de novo* complex rearrangement in the Xp22.1 subdomain with a 517 kb duplications/triplications affecting the first part of *CNKSR2* followed by a 695 kb duplication of the last part of *CNKSR2* and the following four genes (*SMPX*, *MBTPS2*, *SMS* and *PHEX*). It is likely that the *CNKSR2* gene is disrupted by the rearrangement. This boy was born with septooptic dysplasia and no pituitary gland. He was probably moderately mentally retarded until age 6, when idiopathic malignant hyperthermia with rhabdomyolysis caused severe brain damage.
Our findings suggest that mutations in *CNKSR2* could be novel cause of X-linked mental retardation, an assumption supported by high expression in the brain and a role in RAS/MAPK-dependent signal transduction.

P12.148
X-exome sequencing of Finnish families with X-linked intellectual disability

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X-linked intellectual disability (XLID) is present in 2-3% of the population. To date, over 1000 human disorders are known that cause intellectual disability (http://www.ncbi.nlm.nih.gov/Omim/). In half of the cases with severe and in 70 - 80 % of cases with mild ID the cause is unknown. Genetic factors are estimated to cause about 50 % of severe ID. To date, over 90 genes have been identified to underlie XLID (http://xlmr.interfree.it/home.htm).
Here we have applied exome sequencing of the X-chromosome to identify causative genes in four Finnish families with affected males in different generations, suggesting an X-chromosomal mode of inheritance.
In all male patients of the analyzed families a total of 17 variants were predicted. Among them were novel mutations in the established XLID genes *DLG3*, CULB4 and GRIA3. In addition, a splice site mutation in a novel candidate XLID gene was found.
In the preliminary analysis the mutation found in the novel XLID candidate gene is perfectly co-segregating with the disease in the family in which it was found. Further studies are going on to establish the causative nature of the novel gene in XLID. Co-segregation analysis of the mutations in the established XLID genes is in progress.

P12.149
Case story: a boy with fragile X and two microduplications on chromosome Xp.

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We describe a 2 year old boy with delayed motor and cognitive development, and suspicion of progressive muscle weakness. The boy had healthy unrelated parents, and no family history of mental retardation or muscle disease.
The boy was tested and found positive for Fragile X with both PCR and Southern blotting. He had 3 different CGG-repeats on 400, 500 and 900 repeats. The fragile site was inherited from the mother, who had a premutation. Additionally two small duplications were found by array-CGH analysis. Both duplications were located on chromosome X (Xp22.11 and Xp21.2). MLPA-analysis confirmed both duplications. It was found that both duplications were maternally inherited.
The duplication on Xp21.2 involves at least exon 53-67 of the DMD gene, which explains the muscular symptoms. A muscle biopsy showed muscle degeneration with Becker like immune reaction. Duplication within the DMD-gene is relatively well described and the phenotype mostly relates to changes in muscle biopsy.
As to dup Xp22.11 there has been other reports of patients with duplications in the same area, but not of the same size, although there is a strong link to X-linked MR. The extent of MR, is currently too early to evaluate due to the boys young age, but the contribution of dupXp22.11 will be difficult to assess.
In this case we found 3 different mutations that all could contribute to explain the phenotype of this patient. The parents are intending to use this prenatally in the next pregnancy. We are currently awaiting CVS.

P12.150
Retrospective evaluation and prospective routine use of PCR-based protocols for *FMR1* screening and sizing in a clinical setting

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Fragile X is the most common cause of intellectual disability and is inherited via a dynamic expansion of a trinucleotide (CGG) repeat in FMR1. Historically, PCR assays have not adequately resolved large premutations, full mutations, male mosaics, and females with apparent homozygosity. Southern analysis can detect the full mutation, large premutations and evaluate methylation but it does not always detect small premutations. We have performed an evaluation of two protocols that utilize two different sets of *FMR1* PCR primers to establish preliminary evidence of reliability, robustness, sensitivity, specificity and suitability for routine testing. The study was divided into a retrospective part (enriched with expanded allele types) and a prospective (routine, unselected) part. We studied retrospectively DNAs of 200 patients that included a whole range of FMR1 expansion types (normal, grey zone, premutation, full mutation and mosaic samples of both genders). All samples had been previously characterised by in-house PCR and Southern blot. In addition, we studied 330 patients prospectively. Results obtained for prospective patients were verified with in-house PCR and/or Southern blotting. The results obtained were comparable. We were able to detect retrospectively all known expansions and prospectively 25 expanded alleles and reliably determine if female samples were homozygous, heterozygous (resolution of 1 repeat) or carriers of an expanded allele. We propose a workflow for the use of a combination of both sets of primers in a clinical setting and conclude that they can reliably detect all expanded alleles without the use of Southern blotting in female and male samples.

P12.151
Fragile X syndrome molecular diagnosis - 20-years experience from a single laboratory.

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Fragile X syndrome (FXS) is the most common form of inherited X-linked mental retardation. More than 99% of FXS cases are caused by CGG triplet expansion (>200 repeats) in 5' untranslated region of the *FMR1* gene that leads to low expression or absence of the FMRP protein.
Our laboratory is a reference one for FXS diagnosis. Together, 4983 individuals with FXS clinical suspicion and their families were tested with PCR screening method and Southern Blot when necessary. Till 2011, 301 families with confirmed at least one FXS case were registered. Two hundred eighty five probands were diagnosed with *FMR1* mutation. The molecular analysis performed in their families revealed the presence of mutation in 161 relatives (82 males, 79 females) and premutation in 223 relatives (16 males and 207 females, among them 149 mothers).
In 4682 cases (3818 males, 864 females) dynamic mutation in the *FMR1* gene was excluded. The PCR screening method, implemented in 2001, was sufficient for FXS exclusion in 3003 (71.5%) males and 488 (32.2%) females. The implementation of GeneScan method, allowed for the reduction of the number of Southern Blot analyses performed for uninformative cases, from 67.8% to 23.0% ($p < 0,05$) of the total female individuals.

Although new methods are developed for FXS diagnosis, PCR screening with Gene Scan method followed by Southern blot analysis still seems to be the best protocol for this disease. Selected cases with excluded *FMR1* mutation undergo further studies of X-linked mental retardation in our laboratory.

P12.152
Clinical variability in fragile X syndrome

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Fragile X syndrome is the most frequent cause of inherited mental retardation and the second most common cause of genetically associated mental deficiency after trisomy 21.
The majority of fragile X syndrome patients present a loss of function mutation in the FMR1 gene caused by the expansion (>200 repeats) of a single trinucleotide sequence (CGG) located in the FMR1 gene at Xq27.3.
Clinical features in males with fragile X syndrome are represented by developmental delay, intellectual disability, facial dismorphy (long face, large ears, prominent jaw) and macro-orchidism.
The cytogenetic test consists in identifying a "fragile site" on chromosome X from cells cultured in a folate deficient medium. The present standard diagnostic test involves molecular genetic techniques (Southern blot and PCR).
For illustrating the clinical variability in fragile X syndrome, we will present cases from 2 separate families.
The first case, R.D. (4 years old), male, presents cleft palate associated with severe mental retardation, high forehead, elastic decollated ears, mild retrognathism, accentuated plantar creases. The diagnosis was made using PCR; the karyotype did not identify any "fragile site".
The second case is B.D. (27 years old), male, with severe mental retardation, asymmetric elongated face, high and narrow palate, prognathism, big ears, macroorchidism, bilateral thumb hypoplasia, plantar crease. The cytogenetic test identified a 46, XY, fraXq27 karyotype.
The fragile X syndrome is phenotypically heterogeneous. This has important implications for clinical and differential diagnosis emphasizing also the necessity of performing molecular analysis of the FMR1 gene in mentally retarded patients.

P12.153
A new locus for autosomal dominant microcephaly associated with lymphedema

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Microcephaly can occur as an isolated, mostly recessive abnormality or in association with ocular or systemic findings. Microcephaly associated with lymphedema is a rare syndromic variant (MIM 152950) with only few families reported. In some of the latter, ophtalmological findings, including microphthalmia, chorioretinopathy and cataract have been described. Although autosomal recessive inheritance has been suggested in some families, the prevailing hypothesis is that the microcephaly/lymphedema/chorioretinopathy syndrome is a single autosomal dominant entity with variable clinical expression.
Here we ascertained a four-generation family with autosomal dominant microcephaly associated with lymphedema. Lymphedema of hands and feet was apparent at birth but reduced or disappeared spontaneously during childhood in some affected individuals. No distinctive facial dysmorphism was noticed. The affected subjects presented with mild intellectual disability or low normal intelligence. No ophtalmological abnormalities were detected in any of the affected individuals. The proband had a normal male karyotype 46, XY and no copy number variants were detected by array comparative genomic hybridization analysis.
We collected DNA from eleven family members, including five affected subjects, and performed linkage analysis using the Linkage-24 DNA Analysis BeadChip (Illumina) containing 6000 Single Nucleotide Polymorphisms. Data were analyzed with Merlin. We identified a candidate region with a maximum LOD-score of 2.4 on chromosome 10q, measuring 40 MB and containing a total of 492 genes. We will apply whole exome sequencing to identify the responsible gene.

P12.154***
SMOC1 is essential for ocular and limb development in humans and mice

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Microphthalmia with limb anomalies (MLA) is a rare autosomal recessive disorder, presenting with anophthalmia/microphthalmia and hand/foot malformation. We mapped the MLA locus to 14q24 and successfully identified three homozygous (a nonsense and two splice site) mutations in the SPARC (secreted protein acidic and rich in cysteine) related modular calcium binding 1 (*SMOC1*) gene in three families. *Smoc1* is expressed in the developing optic stalk, ventral optic cup and limbs of mouse embryos. *Smoc1* null mice recapitulated MLA phenotypes, including aplasia/hypoplasia of optic nerves, hypoplastic fibula/bowed tibia and syndactyly in limbs. A thinned and irregular ganglion cell layer and atrophy of the anteroventral part of the retina were also observed. Soft tissue syndactyly, resulting from inhibited apoptosis, was related to disturbed expression of genes involved in BMP signaling in the interdigital mesenchyme. Our findings indicate that SMOC1/Smoc1 protein is essential for ocular and limb development in both humans and mice. (Contributing doctors and researchers are really appreciated: Koji Terada, Eliane Chouery, Joelle Abou-Ghoch, Nadine Jalkh, Ferda Ozkinay, Kyoji Horie, Junji Takeda, Tatsuya Furuichi, Shiro Ikegawa, Kiyomi Nishiyama, Satoko Miyatake, Akira Nishimura, Takeshi Mizuguchi, Norio Niikawa, Fumiki Hirahara, Tadashi Kaname, Koh-ichiro Yoshiura, Yoshinori Tsurusaki, Hiroshi Doi, Noriko Miyake, and Takahisa Furukawa.)

P12.155
Common molecular mechanism in homocystinuria: misfolding of cystathionine beta-synthase mutants and effect of chaperones

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Misfolding due to loss-of-function mutations is a common pathogenic mechanism in many genetic diseases including inborn errors of metabolism. To explore the role of misfolding in cystathionine beta-synthase (CBS) deficiency we studied a series of 27 disease-causing mutations located in different domains of the CBS molecule and representing ~70% of patient-derived mutant alleles. Expression in prokaryotic and eukaryotic cells showed a propensity of mutants to misfold/misassemble as evidenced by increased proportion of water-insoluble to water-soluble CBS antigen and decreased formation of correctly assembled tetramers accompanied by impaired catalytic activity (median activities of CBS mutants in *E.coli* and CHO-K1 cells were 3% and 18% of the wild type CBS, respectively; median amounts of tetramers in these expression systems were 12% and 35%, respectively). Proteolytic analysis of a subset of mutants with normal activity and tetramer assembly using thermolysin revealed distinct conformational alterations such as increased unfolding or- in contrast- increased global stability with rigidification. Co-expressional addition of osmolytes glycerol and betaine, and of the heme precursor delta-aminolevulinate to *E.coli* cultures increased activity and formation of tetramers for about one half of mutants; however, addition of phenylbutyrate as a stimulator of heat shock protein expression did not correct misfolding of the series of mutants in CHO-K1 cultures to a significant extent. These studies indicate that about one half of all known patient *CBS* alleles produce enzymes prone to misfolding/misassembly and that correction of misfolding by chaperones may

become an important future therapeutic target .
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P12.156
Mitochondrial gene mutation screening and related whit hearing loss in Hormozgan province in Iran

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Background and aim: Hearing loss is the most frequent sensory disorder occurs in 1/1000 newborns. About 50% of hearin loss cases are due to genetic causes. Mutation in MTRNR1(A1555G), MTTL1(A3243G) and MTTS1(A7445G) are known to be one of the important cause of nonsyndromic Sensorineural hearing loos in some populations.
this study aims to demonstrate the frequency of three mitochondrial mutations including A1555G, A7445G and A3243G in deaf subjects in Hormozgan province in south of Iran.
Method**:** we investigated the presence of three mitochondrial mutations including A1555G, A3243G and A7445G in a cohort of 110 nonsyndromic Sensorineural hearing loos subjects. DNA was extracted using standard phenol -chloroform method. The screening of gene mutations was performed by PCR-RFLP procedure. Finally, the possible mutations were confirmed by direct sequencing.
Results: None of the 110 subjects were found to carry A1555G, A3243G and A7445G mutations. How ever PCR-RFLP of the MTTL1 gene destroyed a restriction site due to G3316A substitution in a deaf subject.
Conclusion: We conclude that the association of A1555G, A3243G and A7445G mutations with hearing loss is very low in Hormozgan 's deaf subjects.

P12.157
Molecular diagnostic - the new face of craniofacial disorders diagnosis

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The great majority of dental health professionals recognized the importance and potential of genetic and genomic evaluations in the future, but little has been done to incorporate this knowledge extensively in clinical practice. More then that, quite a few physicians suggested that dental disorders are common but not lethal for patients. Thus, the subject is not of broad interest to genetic medicine research and is slow-moving field of medical genetics.
Applications of molecular diagnostics in craniofacial disorders will be described. We here report on 2 cases study: cherubism and cleidocranial dysplasia (CCD).
Objectives: The use of DNA samples and PCR assay to test for specific state of disease. The use of molecular genetic testing for diagnostic confirmation when diagnosis of cherubism / CCD is suspected based on clinical and radiological criteria.
Methods: PCR amplification and direct sequencing of the SH3BP2 gene / RUNX2 gene.
Results: Case 1 - of 5 family members, abnormal results were obtained for the patient and her mother. A c.1244G > A mutation was identified in exon 9 of the SH3BP2 gene. Case 2 - of 4 family members, abnormal results were obtained only for the patient. The mutation R225W was identified. A deletion of 7 Glutamines in exon 2 was also identified.**Conclusions**: Both identified mutations are disorder-causing mutations and have a variable expression. We suggest genetic counseling / molecular genetic testing for all patients with such clinical findings, but especially for those with non-familial or sporadic forms.

P12.158***
Identification Of Novel Genes For Autosomal Recessive Intellectual Disability (ARID) In Consanguineous Families

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Identification of the causative genes of autosomal recessive intellectual disability (ARID) has been challenging due to the extreme heterogeneity of the condition. Homozygosity mapping in consanguineous families has been proven to be very helpful in these type of disorders. Here, we have ascertained 60 ARID families from the Pakistani population in which there is a high degree of consanguineous marriages (>70%). Homozygosity mapping data revealed one or more homozygous regions per family ranging in size from 1-51Mb. No pathogenic copy number variations (CNVs) were found. On the basis of homozygosity mapping, we selected seven families with novel ARID loci for targeted massive parallel sequencing (MPS) of the specific homozygous regions, and six families for exome sequencing. Our MPS data revealed a number of potential mutations that are validated at the moment. In one family, PKMR07, this strategy has proven to be successful as we have identified an intragenic homozygous deletion of five exons (11-15) of the *TPO* gene. Mutations of *TPO* are frequent and irreversible cause of ID (if not treated in the early postnatal life), mostly in the developing countries as well as in the entire world. This result confirms that MPS is a powerful method to uncover submicroscopic structural variations that have escaped detection by microarray analysis.

P12.159
Characterization of deletions in Multiple Osteochondromas with arrayCGH

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Hereditary multiple osteochondromas (MO) is an autosomal dominant skeletal disorder characterized by the formation of benign cartilage-capped bone tumours arising from the growth plate area of children's long tubular bones. MO is genetically heterogeneous and is associated with mutations in the tumorsuppressor genes *EXT1* and *EXT2*. Gross *EXT1*/*EXT2*-deletions are responsible for up to 8% of cases. We characterized the breakpoints of such deletions in 10 unrelated MO-patients using an MO-specific tiling-path array, allel-specific PCR-amplification and sequencing analysis.
In 3 families (*EXT1* deletion exon 2-3 (2), *EXT2* deletion exon 8), both deletions breakpoints were identified in homologous sequences of respectively 36bp and 32 bp, located within larger homology regions (~300 bp) which are known Alu-sequence elements. The *EXT2* exon 8-deletion breakpoints were found to be recurrent in 2 families. Both deletions were most likely caused by non-allelic homologous recombination (NAHR).
In the 7 remaining families (*EXT1* deletion exon 2-11 (2), exon 6-7, exon 8, exon 11, *EXT2* deletion exon 2, exon 8), breakpoints were located within short homologous sequences (2-5bp). Consequently, these deletions were assumed to be caused by non-homologous end-joining (NHEJ), in some cases facilitated by the presence of the Alu-sequence elements in the vicinity of one or both breakpoints.
We conclude that gross MO-causing deletions are caused by different mutation mechanisms and that the majority is not recurrent. This study emphasizes once more the high genetic variability characterizing MO.

P12.160
qPCR-HRM applied to *MUTYH* screening : identification of the first large rearrangement in a attenuated familial adenomatous polyposis (AFAP)

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Germline biallelic mutations on the *MUTYH* gene cause one third of attenuated familial adenomatous polyposis (AFAP). This biallelic inactivation is associated with a 28-fold increased risk of colorectal cancer. Despite recurrency in exon 7 and 13, it is more and more recommanded to develop a screening method of the entire gene. Here, we propose to apply the qPCR-HRM strategy as formarly published and show the validation process. The application of this approach reveals a large rearrangement. Material and method: 44 variants with at least one per exon were tested in validation set with 35 heterozygous and 9 homozygous variants. The protocol was designed in duplicate with two master mix with and without template DNA. The quantitative measure was based on delta-deltaCp method. 30 selected patients with attenuated polyposis were also screened. Results: All variants were detected with the HRM curve analysis. 4 homozygous variants (44%) were detected in HRM only with the template DNA mix. In the 30 selected patients, we found 2 homozygous mutations (c.[494A>G]+ [494A>G]) and 2 compound heterozygous mutations (c.[494A>G]+[891+3A>C] c.[1145G>A]+[307_1608del]). Variants and mono-allelic mutations were also identified (n=4). Discussion : The approach qPCR-HRM proposed combining point mutation detection and also large rearrangement identification helps to detect a large deletion (exons 4 to 16) associated to an attenuated polyposis family. This observation expands the spectrum of *MUTYH* alteration. Presently, the frequency of such event is not known. But, it reinforces the need for full screening, particularly in patients with homozygous mutations and with one single heterozygous mutation.

P12.161
Molecular, cellular and clinical heterogeneity of mosaic variegated aneuploidy syndrome

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Mosaic variegated aneuploidy (MVA) syndrome is an autosomal recessive disorder characterised by mosaic aneuploidies, a variety of phenotypic abnormalities and predisposition to cancer. We previously identified biallelic *BUB1B* mutations as a cause of MVA. *BUB1B* encodes BUBR1 which has multiple, crucial roles in the mitotic spindle checkpoint. Our data supported a causal role for aneuploidy in cancer development and exemplifed the insights rare genetic syndromes can provide to our understanding of cancer. However, 60% of cases are not due to *BUB1B* mutations. There is a strong phenotypic overlap between *BUB1B*-positive and *BUB1B*-negative cases but there is a significantly higher risk of cancer in *BUB1B*-positive individuals. Analyses of mitotic checkpoint function demonstrated that the checkpoint defect was profound in *BUB1B*-positive cases. In contrast, the *BUB1B*-negative cases had intermediate or normal checkpoint function. In addition, there was no apparent correlation between the presence or absence of *BUB1B* mutations and the extent of aneuploidies. Taken together, our results clearly highlight the heterogeneous nature of this rare aneuploidy-cancer syndrome. The underlying molecular defect(s) in cases without *BUB1B* mutations have not yet been elucidated. We are currently utilising an exome sequencing strategy to identify further causative gene(s) and this work will also be presented.

P12.162
Genotype-phenotype correlation in cohort of patients with myotonia congenita and frequent CLCN1 gene mutation Arg894Stop

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Myotonia congenita (MC) the most common form of nondystrophic myotonias which can be autosomal dominant or recessive. MC characterized by delayed relaxation of skeletal muscle after voluntary contraction, transitory weakness, skeletal muscle hypertrophy.
Twenty one CLCN1 gene mutations were detected in 33 from 39 patients with MC from MGC, Voronezh. We revealed 5 cases with heterozygous CLCN1 gene mutations and 28 with two mutant alleles. Mutation Arg894Stop was detected in 19 from 61 mutant chromosomes (31%). It is interesting to note a wide variety of clinical presentations in cohort of patients with detected Arg894Stop: from phenotypes ranging from pronounced myotonia to such mild symptoms that myotonia was only revealed upon careful clinical examination.
Neurophysiologically, considerable decrement (69%) of compound muscle action potential (CMAP) was revealed in all patients with Arg894Stop during high frequency repetitive nerve stimulation (50Hz for 200 stimuli). We marked correlation of allelic state of Arg894Stop with amplitude of CMAP. Posttetanic transitory depression (PTD) (after 50 Hg) of CMAP considerable fluctuates (43%) in all our MC patients. In compound heterozygous (Arg894Stop/...) PTD of CMAP decreased from 11,2±3,3mV to 7,1±5,5mV (37%). There is no PTD of CMAP in heterozygous and homozygous patients. PTD correlations allow to estimate efficiency of restoration CLCN1 function in patients with different state of mutant alleles with Arg894Stop and to optimize molecular-genetic diagnostic of MC patients.

P12.163
Simultaneous multiplex PCR based myotonic dystrophy type 1 and type 2 molecular testing

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Myotonic dystrophy type 1 (DM1) is caused by expansion of a (CTG) n repeat in the DMPK gene, while myotonic dystrophy type 2 (DM2) is caused by expansion of a (CCTG)n part of a complex repetitive motif (TG)n(TCTG)n(CCTG)n. The size of healthy range alleles is highly variable in both loci and expanded alleles may contain several thousands of repeats in both DM1 and DM2. Combination of conventional PCR and “triplet” or “tetraplet” repeat-primed PCR (TP-PCR) is a commonly used approach to characterize healthy range alleles and to prove the presence or absence of expanded alleles in molecular testing for both disorders. We have designed three multiplex PCR reactions for simultaneous characterisation of the DM1 and DM2 locus using PCR based analyses and capillary electrophoresis on automatic DNA analyser. The first reaction includes bi-directionally labelled conventional PCRs for both the DM1 and DM2 locus. The second reaction contains the reverse directional DM1 TP-PCR and the reverse directional DM2 TP-PCR, while the third reaction was designed to include the forward directional DM1 TP-PCR and the forward directional DM2 TP-PCR. These three reactions were designed to be performable under the same amplification and electrophoretic conditions allowing thus their parallelisation. The usefulness of our multiplex assay is underlined by suggestions from previous studies, which showed, that performing conventional PCR with bi-directional labelling of specific amplicons and TP-PCRs simultaneously from both sides of the repeat regions can increase the reliability and accuracy of the assays which are based on the described methods.

P12.164
A canine *Arylsulfatase G* (*ARSG*) mutation leading to a sulfatase deficiency is associated with neuronal ceroid lipofuscinosis

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Neuronal ceroid lipofuscinoses (NCLs) constitute the most common group of inherited children's progressive encephalopathies, characterized by progressive loss of vision, mental or motor deterioration, epileptic seizures and premature death. All NCLs, but rare autosomal dominant adult-onset forms, segregate as autosomal recessive morbidity traits and are classified into 10 genetic forms designated CLN1 to CLN10. Eight of them (CLN1-3, CLN5-8 and CLN10) were molecularly characterized. Genes underlying CLN9 and adult-onset forms, known as Kufs' disease (CLN4), remained to be identified.

A spontaneous canine model of adult American Staffordshire Terriers developping a late-onset cerebellar abiotrophy with Purkinje cell loss has been previously described and we confirmed that US and French affected dogs suffer from an autosomal recessive adult-onset form of NCL. Through a combined genome-wide association and linkage study, we identified a polymorphism in exon 2 of the *Arylsulfatase G* (*ARSG*) gene, encoding a lysosomal sulfatase. The polymorphism caused a non-conservative p.R99H substitution in the protein. The missense change, in the vicinity of the ARSG catalytic domain, lead to a 75% sulfatase activity decrease in leucocytes from affected dogs, confirming at the functional level that the variant may be the NCL-causing mutation (PNAS 2010, vol 107, 14775-14780). Our results unraveled a key role, presently evaluated in human patients affected by NCL, played by ARSG in NCL and neuronal homeostasis. Additionally, collection of fully phenotyped affected dogs is ongoing and should help identify modifying genes that may be further considered as hard candidates for NCL pathogenesis.

P12.165
Clinical and molecular characterization of fifteen patients with six novel mutations causing Nucleotide Excision Repair (NER) related diseases.

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Nucleotide Excision Repair (NER) is one of the major DNA repair mechanisms responsible for repairing DNA damaged by either UV-irradiation or by certain chemicals that form "bulky" DNA adducts. The NER pathway involves at least 28 genes. Mutations in 13 NER genes have been associated with Xeroderma pigmentosum (XP), Cockayne syndrome (CS) and Trichothiodystrophy (TTD), with complex and varying multisystem pathologies.

These diseases are rare worldwide, but are frequent in Israel and the Middle East, probably due to the high rate of consanguinity among certain populations.

The genotype phenotype correlation in those diseases has not been fully clarified. Each clinical phenotype can arise from mutations in more than one gene and conversely, different mutations in one gene can give rise to more than one clinical phenotype.

We have clinically characterized fifteen patients with NER defects and identified their causative genes and mutations. Our analyses revealed 4 XP patients, 8 CS patients, 1 XP/CS patient and 2 TTD patients. We have identified 6 novel mutations in *CSB, XPD, and XPG*. We have established a population screening program for CS in one of these villages where the carrier frequency was found to be 1:9.

Our data might contribute to the understanding of the phenotype genotype relationship and basic pathology of NER related diseases. Identification of the causative genes allows us to establish accurate genetic counseling and molecular diagnosis to couples at risk and high risk populations.

P12.166
Ketogenic diet accelerates the onset of disease in a PLA2G6 KO mouse model

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A mouse model of neuroaxonal dystrophy, with targeted disruption of PLA2G6 gene (KO), characterized by a late onset and slow progression of the disease, was described by Shinzawa K. et al in 2008. In order to accelerate the onset of pathology we decided to stress genotype and lipid metabolism of these mice using a high-fat ketogenic diet (KD).

After 60 days of KD diet KO mice developed diffuse alopecia, hypotonia of dorsal muscles with hunched posture and several motor dysfunction that not appear in control animals.

KO animals showed a significant loss of endurance on treadmill test and a reduced stride length on foot print test. Autopsy revealed the presence of fat liver in KO but not in control animals.

Electron microscopy analysis of sciatic nerve ultrastructure was normal in control sample, whereas in the KO mouse disclosed the presence of Wallerian-like degeneration in different phases of evolution and a large number of myelinated fibres characterized by cytoplasmic accumulation of vesicular and tubulo-vesicular structures. Also un-myelinated fibres showed degenerative features and the presence of some swollen fibres resembling spheroid-like structures.

Histochemistry revealed a strong reduction of COX (cytochrome c oxidase) activity in peripheral nerve, a partial reduction in the muscle with some COX-negative fibres, and no deficiency in the brain. In brain cortex we also observed some apoptotic Tunel positive nuclei.

The KD diet is able to accelerate onset and disease progression, and to unravel a biochemical phenotype not present in PLA2G6 KO mice fed with standard diet.

P12.167
NF1 and SPRED1 molecular analysis: Experience of a french NF Center.

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The identification of mutations in the NF1 gene causing type 1 neurofibromatosis (NF1 - OMIM 162200) is still presenting a considerable amount of work mainly because of the large size of the gene and the restricted number of recurent mutations. The high frequency of NF1 which lead us to choose two complementary methods for NF1 gene analysis:

- the multiplex ligation-dependant probe amplification (MLPA P081, P082, P122C1)
- and the automated denaturing high performance liquid (dHPLC) screening method.

The MLPA method was validated by the detection of 19 known large NF1 gene deletions.

The dHPLC was optimised for a rapid screening of the 60 exons and the splice junctions of the NF1 gene. The sensitivity was evaluated in a MLPA/dHPLC analysis of a panel of 150 unrelated french NF1 patients.

Seven large deletions were first detected by P081/082 MLPA. Mutations were identified in 136 among the 143 patients left with a global mutation detection rate of 96% [CI95%: 91-98].

Then, the seven NF1 negative patients were associated to nine others adult patients with a mild NF1 phenotype (pigmentary changes but no neurofibroma) to be screened by direct sequencing for mutations of the SPRED1 gene. SPRED1 mutations were identified in 2 cases compatible with Legius syndrome phenotype.

Our results confirm that the association of the MLPA and dHPLC techniques provides an accurate and fast method for the identification of NF1 mutations

The results show also the interest of the SPRED1 analysis and the implication for the counselling of NF1 families.

P12.168
Characterization of the molecular mechanisms underlying atypical NF1 deletions

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Large deletions encompassing the NF1 gene and its flanking regions constitute the most frequent recurrent mutations causing neurofibromatosis type-1 (NF1). Four types of large NF1 deletion (type-1, type-2, type-3 and atypical) have been identified to date. These differ with respect to the extent of the deleted region, the location of the respective breakpoints and the underlying causative mechanisms. Whereas type-1, type-2 and type-3 NF1 deletions are mediated by NAHR between low-copy repeats, atypical NF1 deletions appear to have non-recurrent breakpoints and differ quite markedly in terms of their extent.

In this study, we have collected a cohort of 16 patients with de novo atypical NF1 deletions. Our aim was to precisely map the deletion breakpoints and to characterize the breakpoint-flanking sequences in order to identify the mechanism(s) underlying these atypical deletions. Initial analysis using MLPA and PCR-based breakpoint mapping using somatic cell hybrids carrying only the chromosome 17 with the deletion of the respective patients generated several important findings: First, non-homologous-end-joining (NHEJ) is the major mechanism responsible for atypical NF1 deletions. Second, one of both deletion breakpoints maps to one of the LCRs located in the NF1 gene region. These observations imply that NHEJ is driven by the local genomic architecture, specifically the LCRs in the NF1 gene region. Our findings indicate that the impact of the repetitivity of the LCRs located in the NF1 gene region is not confined to replication-based mutational mechanisms such as FoSTeS and MMBIR but is also evident in the context of recombination-based NHEJ events.

P12.169
Epidemiological and molecular genetic study of Neurofibromatosis type 1 in the Republic of Bashkortostan

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Neurofibromatosis type 1 (NF1) is a common autosomal dominant genetic disorder that affects 1 in 3500 individuals worldwide, in which affected individuals develop benign and malignant nerve tumors. This inherited disorder is due to germline mutations of the *NF1* gene, a 17q11.2-located gene that spans 280 kb, is composed of 61 exons.

In Bashkortostan Republic according to January 2011 revealed 245 NF1 patients from 196 families. The prevalence of the disease is 4.7 per 100 thousand population. 55% of NF1 disease are sporadic cases. DNA of 85 NF1 patients (66 families) was extracted from blood. All the patients were residents of Bashkortostan Republic. Currently we identified five mutations in 6 patients from 5 families, 4 of which were sporadic cases. We found: two missense mutations: c.733T>A C245S, c.2990 G>C R997W; 2 nonsense mutations: c.1278 G>A W426X, 4537 C>T R1514X; 1 splice site mutation c.4514+5 G>A 4646+5G>A. All identified mutations haven't been previously described. Also revealed two polymorphisms: A2877G (Gln) and T2898C (Ala) in 5 patients from 5 families.

In patients with identified missense and nonsense mutations the disease was characterized by multiple neurofibromas and pigmented spots on the body; in a patient with identified splice site mutation (sporadic case) neurofibromas was focal - single, spots all over his body.

P12.170
Analysis of the *NF2* gene in a cohort of 16 unselected patients suspected of having neurofibromatosis type 2

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Neurofibromatosis Type 2 (NF2) [MIM 101000] is an autosomal dominant disorder that is characterized by nervous system tumours and ocular abnormalities, affecting 1 in 40,000 individuals. The disease is caused by mutations in the *NF2* tumour suppressor gene, at 22q11.2.

The distinguishing clinical feature of NF2 is the presence of bilateral vestibular schwannomas. Patients with NF2 also develop schwannomas at other locations and other tumours such as meningiomas, gliomas and neurofibromas. NF2 should be distinguished from neurofibromatosis type 1 (NF1) and from schwannomatosis. Whereas neurofibromas are the cardinal manifestation of NF1, patients with schwannomatosis develop multiple schwannomas affecting the peripheral nerves and spinal nerve roots without vestibular schwannomas or other NF2 related tumours.

We screened for mutations in the *NF2* gene a panel of 16 unrelated patients referred to our laboratory because of clinical features that required the consideration of NF2, using direct cDNA sequencing complemented with MLPA analysis.

Possible disease causing mutations were identified in 3 (18%) cases. They consist in a 3'UTR reported sequence variant of unknown biological significance and two splice errors, one of them identified in a patient with bilateral vestibular schwannomas and a spinal astrocytoma. The 13 patients without any identified *NF2* mutation had a wide variety of nervous system tumours, but just one was suspected of having bilateral vestibular schwannomas.

Our experience evidences that diagnosis of NF2 is often difficult to make when based only on clinical characteristics. In this context, genetic testing helps clinicians to establish a more precise diagnosis.

P12.171
Novel and recurrent mutations in NF1 gene - the first genetic study in Croatian patients with neurofibromatosis type 1

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Background: Neurofibromatosis type 1 (NF1) is one of the commonest autosomal dominant disorders in man. The clinical manifestations of disease are diverse, heterogeneous in origin, and age-dependent. The clinical diagnosis of NF1 is based on the presence of clinical criteria developed by National Institutes of Health Consensus Conference. To date, a number of different novel and recurrent mutations of NF1gene have been found.

The aim of this study was to identify the spectrum of disease causing mutations in the NF1 gene in Croatian non-related patients who fulfilled only one NIH diagnostic criterion at the time of NF1 gene testing. All children with sporadic NF1 are continued to be followed-up prospectively.

Results: Of 21 NF1 gene analyses, 18 (85.71 %) were positive. In 16/21 patients the specific mutations were found by gene sequencing and in two children MLPA revealed the deletion of whole NF1 gene. Thirteen of 16 found NF1 mutations were novel: c.147C>G, c.205-2A>C (IVS2-2A>C), c.1260+1G>C (IVS9+1G>C), c.1393_1421dup, c.1659_1660dupTC, c.3144G>A, c.3445A>G, c.4076delC, c.4168C>T, c.4168C>T, c.4686delA and c.4756dupT, while 3 mutations have been previously identified: c.1721G>A, c.3827G>A, c.3827G>A. No mutational hot spots in NF1 gene were observed. Among NF1 patients we reported two monozygotic twins with c.4168C>T mutation and concordant clinical features so far.

Conclusion: The mutational spectrum showed mainly nucleotide substitutions, suggesting that the genotypic characteristics of Croatian NF1 patients may be distinct from that of other populations. We present this study as the first step in the routine diagnosis procedure for Croatian patients with NF1.

P12.172
Loss of PAK3 kinase activity underlies a novel form of X- linked neuroichthyosis

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We report the identification of a gene potentially responsible for a novel X-linked form of syndromic neuroichthyosis, segregating in a family characterized by ichthyosis, corpus callosum agenesis, microcephalia, seizures, mental retardation and spastic tetraparesis.
Linkage analysis revealed a critical region of nearly 25 Mb in Xq21.33q24 including about 250 genes. Having observed a clinical overlap with the cardio-facio-cutaneous syndrome, we analyzed the genomic sequence of *PAK3*, a gene involved in K-ras pathway, coding for a serin/threonine kinase with an important role in the organization of cytoskeleton. We found the K389N substitution within the highly conserved kinase domain of the protein. This mutation segregated with the disease in the family and it has never been detected in more than 450 control chromosomes; moreover prediction tools described this aminoacidic substitution as probably damaging.
As mutations in *PAK3* have been already reported in association with isolated mental retardation (MR), we compared the kinase activity of the wild-type, the K389N and the MR forms, showing a complete loss of function for all mutants.
These data suggest a causative role of the K389N mutation in the pathogenesis of neuroichthyosis, although the underlying mechanism leading to a complex disease is not clear.
We can not exclude that another gene within the critical region could be involved, worsening the clinical picture caused by PAK3 mutations, but the relevant expression we observed in keratynocytes would suggest an implication of this gene also in the ichthyosis occurrence, possibly due to additional kinase-independent activities of PAK3.

P12.173
Next Generation Sequencing for the Analysis of Monogenic Traits

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Next Generation Sequencing (NGS) technology has opened up new avenues for the clarification of gene variants causing Mendelian disorders. Most monogenic disorders are caused by either exonic sequence variants or splice-site mutations. These variants can now be readily identified by sequencing all exons in the human genome (exome sequencing). However, identification of a disease causing variant is not trivial due to the extensive number of rare variants identified in individual exomes. Although databases such as dbSNP can be used to reduce the candidates by filtering out common variants, the remaining candidates are still often numerous.
We present here the results from exome-sequencing in five individuals of Pakistani origin affected by various monogenic disorders. We identified approximately 85,000 variants (synonymous and non-synonymous) in each individual. After database filtering on average over 12,000 variants could be described as novel and 3,300 of these are exonic. The majority of the novel exonic SNPs were non-synonymous (70%) whereas 3% were nonsense variations and 1.5% were splice site alterations. Furthermore, each individual carries on average six nonsense variants in a homozygous state and about 20 in a compound heterozygous state together with a novel nonsynonymous or nonsense variant.
Our findings illustrate the extensive number of rare and possibly disease causing variants that can be identified by exome sequencing in any individual. The main challenge using NGS sequencing for disease gene tracking is to differentiate between the many rare benign variants and disease causing mutations. Strategies to overcome this problem will be presented in more detail.

P12.174
Molecular Analysis of GJB2, GJB6 Genes and A1555G Mitochondrial Point mutation in Patients with Nonsyndromic Hearing Loss, the Italian Job.

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Mutations in the *GJB2* gene, which encodes the gap-junction protein Connexin 26 (Cx26), are the most common cause of nonsyndromic hearing loss (NSHL) accounting for about 32% of cases.
The subjects referred to our North Italian Centre are affected by sensory neural deafness with various degrees of hearing loss. Since January 2001 we analyzed 1250 patients and identified mutations in 763/2500 chromosomes. We characterized more than 40 different mutations and nine polymorphisms in 435 NSHL subjects. Our data confirm 35delG as the most frequent *GJB2* mutation in the Italian population accounting for about 64% of all the Cx26 alleles we identified. In recent years we also observed in our cases an increase of the most frequent polymorphisms in Asiatic and South American populations. The *GJB6* gene deletion, del(*GJB6*-D13S1830), was identified in seven patients carrying a *GJB2* mutation in the other allele and in homozygote status in one subject, while the del(*GJB6*-D13S1854) was not observed in our cohort of patients. Moreover, 45 affected subjects were compound heterozygous for recessive GJB2 allele not including 35delG and 8 were carrying dominant mutations (T55N, P58A, D179N and R184Q), indicating that the complete sequence of the gene is needed for an appropriate molecular diagnosis. The analysis of the deafness-causing A1555G substitution in MTRNR1 mitochondrial gene was carried out in patients without Cx26 recessive mutations. We found 24 affected subjects carrying the A1555G and the subsequently family analysis performed in each case has led to the pre-symptomatic identification of this mutation in relatives.

P12.175
Spectrum of *CLCN1* and *SCN4A* mutations in Czech patients with non-dystrophic myotonias.

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The non-dystrophic myotonias are a heterogeneous group of rare inherited diseases that demonstrate myotonia as their common feature, in reference to a delayed muscle relaxation after voluntary or evoked muscle contraction. The non-dystrophic myotonias are skeletal muscle ion channel diseases which are caused by mutations in the genes coding for the skeletal muscle chloride channel 1 (*CLCN1*) or the alpha subunit of voltage-gated sodium channel 4 (*SCN4A*).
Mutations of the *CLCN1* gene result in either autosomal dominant myotonia congenita (Thomsen type) or autosomal recessive myotonia congenita (Becker type). A subset of *CLCN1* mutations have been found to cause both recessive and dominant myotonia. Mutations of

the *SCN4A* gene are typically inherited as an autosomal dominant trait, regardless of the associated phenotype (paramyotonia congenita, hyperkalemic periodic paralysis, hypokalemic periodic paralysis, normokalemic periodic paralysis, potassium-aggravated myotonia, myotonia fluctuans, and congenital myasthenic syndrome).
Molecular-genetic diagnostics of non-dystrophic myotonias was initiated in our laboratory in 2009. Since then, DNA diagnostics was performed in 55 unrelated patients suspected to be myotonia congenita, and confirmed in 41 of them. Mutations in the *CLCN1* gene were detected in 28 patients (25 with recessive and 3 with dominant myotonia congenita), mutation in the *SCN4A* gene in 13 patients. We identified 20 types of *CLCN1* mutations (6 mutations were novel not described previously) and 6 types of *SCN4A* mutations, (3 mutations were novel).
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P12.176
Twelve-years investigation of DFNB1 (*GJB2*) mutations in Iranian population with non-syndromic hearing loss

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DFNB1 locus has been recognized to account for a large proportion of cases of non-syndromic autosomal recessive deafness and the gene that encodes connexin 26, *GJB2*, has been identified as causative in DFNB1. The *GJB2* gene is also involved in an autosomal dominant form of deafness, DFNA3. In present study, we investigate the prevalence of *GJB2* mutations in Iranian deaf population with different ethnic groups. Two thousand three hundred thirty individuals, who were referred to GRC, were subjected to *GJB2* screening. The study was approved by the regional ethics committee of the USWR for medical research. After signing consent form by all participants and clinical examination, patients were screened for 35delG, the most prevalent *GJB2* mutation. Further analysis was performed for normal or heterozygote individuals, by sequencing of *GJB2*-exon 2, as well as screening of exon1 for all heterozygote families. Total of 370 (~16%) families were affected with *GJB2*-related deafness including both recessive and dominant mutations in homozygote and compound heterozygote forms. Forty five different *GJB2* mutations including two novel mutations have been identified. *GJB2* mutations in both alleles have been found in 89% of chromosomes. However, 35delG was the most common *GJB2* mutation accounting for 64% of the mutations in population studied. Distribution of *GJB2* mutations varied in different parts of Iran. Our data suggest that there is a gradual decrease in the frequency of the 35delG mutation and *GJB2*-related deafness in general as we move from the Northwest to South and East through the Persian Gulf countries.

P12.177
First description of M504A mutation in PTPN11 gene detected in a spanish patient with noonan syndrome

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BACKGROUND: Noonan syndrome (OMIM 163950) is a rare disease, mainly presenting with malformations such as dysplasia and stenosis of the pulmonary valve, atrial septal defect and a typical pattern of hypertrophic cardiomyopathy. It may be sporadic or inherited as an autosomal dominant or recessive trait and occurs in 1 in 1000-2500 children. In more than 50% of patients with Noonan syndrome, mutations in the *PTPN11* gene have been identified. This gene encodes a tyrosine-protein phosphatase non-receptor type II (SHP-2).
CASE REPORT: We report the case of a 9-year-old girl. This patient was diagnosed at birth with severe pulmonary stenosis with a dysplastic valve, and mild supravalvular stenosis. She presented with a permeable oval foramen. A valvuloplasty was performed at 2 years of age with limited results due to supravalvular obstruction and the type of valve. As her phenotype presented characteristics that were typical of Noonan syndrome, we studied the *PTPN11* gene by automatic sequencing of all exons and intron boundaries.
CONCLUSIONS: The patient was heterozygous for the M504A mutation (c.1510A>G, p.Met504Val) in exon 13 of the *PTPN11* gene. This mutation is located in the PTP domain of PTPN11 and is in close spatial proximity to important residues that stabilize the protein. The pathogenic mechanism in Noonan syndrome involves altered N-SH2/PTP interactions that destabilize the inactive conformation without altering the SHP-2 catalytic capability. PTPN11 is a key molecule in the cellular response to growth factors, hormones, cytokines, and cell adhesion molecules. This is the first time that the M504A mutation has been reported in the Spanish population.

P12.178
Heterozygous germline mutations in the *CBL* tumor-suppressor gene cause a Noonan syndrome-like phenotype

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RAS signaling plays a key role in controlling appropriate cell responses to extracellular stimuli, and participates in early and late developmental processes. While enhanced flow through this pathway had been established as a major contributor to oncogenesis, recent discoveries have revealed that aberrant RAS activation causes a group of clinically related disorders characterized by facial dysmorphism, a wide spectrum of cardiac disease, reduced growth, variable cognitive deficits, ectodermal and musculoskeletal anomalies, and increased risk for certain malignancies. Here, we report that heterozygous germline mutations in *CBL*, a tumor-suppressor gene encoding for a multivalent adaptor protein with E3 ubiquitin ligase activity mutated in myeloid malignancies, can underlie a phenotype with clinical features fitting or partially overlapping Noonan syndrome (NS), the most common condition of this disease family. *CBL* mutations were identified in four sporadic cases and two families from among 365 unrelated subjects with NS or suggestive features and negative for mutations in previously identified disease genes. Phenotypic heterogeneity and variable expressivity were documented. Four mutations were missense changes affecting the RING finger domain or the linker connecting this domain to the *N*-terminal tyrosine kinase binding domain. One splice-site nucleotide substitution, resulting in skipping of the exon coding for those domains, and one truncating mutation at the *N*-terminus were also identified. Mutations were shown to affect CBL-mediated receptor ubiquitylation and dysregulate signal flow through RAS. These findings document that germline mutations in *CBL* alter development to cause a clinically variable condition that resembles NS and that possibly predisposes to malignancies.

P12.179
Noonan Syndrome diagnosed by custom molecular microarray (Array CGC)

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Introduction: Noonan syndrome is a congenital genetic disease that affects both males and females equally (1:1.000-2.500 newborns). Great clinical variability has been identified: heart disease, valvular pulmonary stenosis or hypertrophic cardiomyopathy, short stature of

postnatal onset, psychomotor development delay. Often this syndrome is not diagnosed, but it is related to many complex problems such as coagulation defects and lymphatic dysplasias. Differential diagnosis includes major diseases in the same metabolic pathway: Costello, Cardiofaciocutaneous and LEOPARD Syndrome.
Method: With a custom microarray panel (Array CGC - Pat. Pending) that contains a panel of 80 point mutations, in 8 main genes involved in Noonan (5 genes) and other genetically related syndromes (PTPN11, RAF1, SOS1, KRAS, HRAS, BRAF, MAP2K1 and MAP2K2) is possible to identify the molecular basis of the most frequent and severe forms.
Results: Using the array panel we analyzed 6 cases of Noonan syndrome, where mutations on the PTPN11, were detected by sequence analysis. All the mutations were also detected with the array. Additionally, 6 cases with clinical suspicion and a PTPN11 negative sequence analysis were also studied. In one case a mutation on the SOS1 gene was detected, making the molecular diagnosis possible.
Conclusion: This approach is an useful and valuable tool for diagnostics, since it detects the most common mutations associated with Noonan Syndrome, not only on PTPN11 gene but also in 4 other genes, improving the capacity for diagnosis. A broader clinical spectrum and a faster diagnosis are achieved, allowing an earlier decision-making process in patient management.

P12.180
Mutation analysis of the genes involved in the Ras-MAPK pathway in Polish patients with Noonan syndrome

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Noonan syndrome (NS) is an autosomal dominant disorder characterized mainly by short stature, congenital heart defects, facial dysmorphism and chest deformities. Heterozygous gain of function mutations in various genes encoding proteins of the Ras-MAPK signaling pathway have been identified as the genetic basis of NS. Although NS is relatively commonly inherited disorder (1:1000-1:2500 live births), it has been molecularly characterized in only a small proportion of human populations throughout the world and there are no reports on the genetic background of Slavic patients with NS. Here we report the results of molecular and clinical analysis in a cohort of 59 patients affected by NS. Mutation analysis of PTPN11, SOS1, RAF1 and KRAS genes confirmed NS in 56 patients, including 40 probands and 16 affected relatives, with a total mutation detection rate of 67.8%. We identified missense mutations in PTPN11 for 28, SOS1 for 10 and RAF1 for 2 unrelated patients. All of the changes were already known, except for two novel substitutions in SOS1 and one in RAF1 gene. The correlation between genotype and phenotype is similar to that observed in other studies. Patients with SOS1 mutations showed higher frequency of macrocephaly, ptosis, webbed neck, mitral valve anomaly, renal and ectodermal abnormalities in comparison to the group with mutations in PTPN11. This study demonstrated that only three most common genes related to Ras-MAPK pathway (PTPN11, SOS1, RAF1) are involved in the pathogenesis of Polish NS patients.
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P12.181
Novel sequence changes in the NOTCH1 gene in patients with bicuspid aortic valves

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Bicuspid aortic valve (BAV) is the most common congenital cardiac malformation occurring in 1-2% of the population. BAV is associated with significantly increased risk of developing thoracic aortic aneurysm and acute aortic dissection. It was shown that genotypic variations in the *NOTCH1* gene are associated with BAV and may contribute to the observed variability in aortic phenotype. We performed *NOTCH1* mutational analysis in 30 unrelated subjects with BAV and 20 unrelated controls from general Czech population using high resolution melting analysis and DNA sequencing. We focused preferentially on exons in which mutations associated with BAV have already been reported. In general population we detected one sequence change within exon and two intron variants which have not been described yet. The variant p.Asp1026Asn was found in one control sample and causes an amino acid substitution. The variant c.2587+20GtoA was detected in one sample from general population and also two patients with BAV. Its effect on alternative splicing of mRNA cannot be excluded. In addition, we discovered two novel sequence variants within exons in the patient group. The variant p.Cys570Arg causes amino acid substitution and probably disrupts a disulphide bond in the extracellular receptor domain. Another variant p.Cys144Cys is a silent mutation probably without a functional significance. Further studies are necessary to elucidate the potential role of detected changes in disease manifestation. In general, identification of aneurysm-predisposing susceptibility genes may lead to gene-directed surgical therapy for patients with BAV in the future.
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P12.182
Cerebral dysgenesis does not exclude OFD I syndrome

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Oral-facial-digital syndromes (OFD) are defined by the association of malformations involving the mouth, the face and the digits, with different modes of inheritance. Other organ systems can be involved, defining 13 specific subtypes. CNS malformations appear particularly associated with OFD I and VI syndromes. In OFD I syndrome, while corpus callosum agenesis appears common (40%), other malformations, including intracerebral arachnoid cysts, porencephaly, heterotopias, cerebellar malformations, and abnormal gyrations, are rarely described. Only molecular bases of the OFD I syndrome have been elucidated, when the OFD1 gene has been identified. However, mutations have also been identified in the TMEM216 gene in two patients with OFD VI syndrome and in the GLI3 gene in five patients with OFD syndrome and median anomalies. Few years ago, Lesca et al. [2006] described an OFD syndrome with cerebellar dysgenesis involving all the brain structures in a sporadic female foetus. We recently identified a causal c.400_403delGAAA mutation the OFD1 gene. This mutation has previously been reported in two sporadic cases with OFD I syndrome and normal neurological examination. The X chromosome inactivation pattern studied in three fetal tissues showed skewed X inactivation (0-100%) in all tissues. The identification of an OFD1 mutation permit to reclassify this patient as OFD I, instead of a new cerebral dysgenesis type as proposed by the authors. It illustrates also the high clinical variability in CNS features in OFD I syndrome. Therefore, sporadic severe CNS malformation associated or not with cerebellar findings does not exclude primarily OFD I syndrome.

P12.183
Multiplex ligation-dependent probe amplification (MLPA) in the diagnosis of 1p/19q deletions in oligodendroglial tumours.

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The combined loss of the short arm of chromosome 1 and the long arm of chromosome 19 is a molecular signature strongly associated with oligodendroglial tumours. Gliomas with a 1p/19q codeletion frequently respond favourably to chemotherapy and are associated with longer survival times. To date, the detection of 1p/19q loss has been most frequently carried out by fluorescent in-situ hybridisation or by loss of

heterozygosity testing. Recently, multiplex ligation-dependent probe amplification (MLPA) has been developed as an efficient, sensitive and specific alternative to these tests. This work describes the development and validation of the MLPA method for detection of 1p/19q loss in paraffin sections from glioma samples at St George's.
A total of 53 brain tumours were tested by MLPA for the 1p/19q deletions using the kit P088-B1 from MRC Holland: 5 glioblastomas with oligodendroglioma component (WHO grade IV); 19 anaplastic oligodendrogliomas (WHO grade III); 19 oligodendrogliomas (WHO grade II); 1 anaplastic oligoastrocytoma (WHO grade III); 4 oligoastrocytomas (WHO grade II); 2 anaplastic ependymomas (WHO grade III); 2 pilocytic astrocytomas (WHO grade I) and 1 central neurocytoma (WHO grade I). A comparison between MLPA and FISH is provided for 23 of the cases.
1p/19q codeletion was detected in 79% of the pure oligodendroglial tumours (WHO grades II and III) and 40% of the oligoastrocytomas (WHO grades II and III). Other results will be further discussed. Over 90% of MLPA and FISH results were concordant.
MLPA provides a cost effective and reliable method for diagnostic detection of the 1p/19q codeletion.

P12.184
OPA1 mutation in family with syndromic form of DOA from Russia

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Mutations in OPA1, a dynamin-related GTPase involved in mitochondrial fusion, cristae organization and control of apoptosis, have been linked to non-syndromic optic neuropathy transmitted as an autosomal-dominant trait (DOA OMIM#165500). Here we report about four patients from three generation family with syndromic form DOA associated with sensoneural deafness, chronic progressive external ophthalmoplegia, ataxia, axonal sensory-motor polyneuropathy.
Proband is a 39-year-old woman with optic atrophy manifestation at the age of 10 years and with moderate sensorineural hearing impairment since the age of 22 years. At a recent neurological examination she showed bilateral ptosis, external ophthalmoplegia, severe optic atrophy and deafness and had an ataxic gait with positive Romberg sign. Neurophysiological studies suggested that deafness was caused by auditive neuropathy. Nerve conduction studies revealed an axonal sensorimotor neuropathy, without additional myopathic features on needle electromyography. Brain MR imaging revealed a number of small high-signal lesions in the frontal and temporal white matter on T2 imaging, thought to represent focal ischemic lesions. Younger brother, sister and nephew had similar symptoms in heavier form. The disease current was slowly progressing.
OPA1 gene was amplified by PCR with specific primers designed to amplify all exons and flanking intronic regions. All four affected patients with optic atrophy 'plus' clinical phenotypes underwent complete sequence analysis of the OPA1 gene. The c.1334 G > A (p.R445H) mutation in exon 14 caused disease in this family. This mutation introduce amino-acid changes in highly conserved positions of the GTPase domain.

P12.185
Production of a mutant oligophrenin 1 protein causes mental retardation and cerebellar hypoplasia in a three generation Italian family.

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A three-generation family with two maternal cousins and an uncle affected by moderate to severe mental retardation and strabismus was referred to our clinic. One cousin presented during infancy with hydrocephalus and cerebellar hypoplasia, while the other cousin displayed marked hypoplasia of the cerebellar vermis and asymmetric hypoplasia of cerebellar lobes. The X-linked inheritance and the presence of cerebellar malformation suggested a mutation in the OPHN1 gene and mutational screening revealed a 2-bp deletion that abolishes a donor splicing site, resulting in the inclusion of 48-bp tract of intron 7. This mutation leads to the production of a mutant oligophrenin 1 protein with 16 extra aminoacids inserted in-frame in the N-terminal BAR domain. To our knowledge this is the first case of a mutation in OPHN1 that does not result in the production of a truncated protein or in its complete loss. OPHN1 (ARHGAP41) encodes a GTPase activating protein belonging to the GRAF proteins subfamily characterized by an N-terminal BAR domain, followed by a pleckstrin-homology domain and the GAP domain. GRAF proteins play a role in endocytosis and are supposed to dimerize via their BAR domain, that induces membrane curvature. The extra 16 aminoacids cause the insertion of 4.4 turns in the third alpha-helix of the BAR domain, which seems to impair the protein function. In fact, the clinical phenotype of these patients is identical to that of patients with loss-of-function mutations.

P12.186
Mutation and haplotype analysis of oculopharyngeal muscular dystrophy in Yakut population

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Oculopharyngeal muscular dystrophy (OPMD) is an inherited neuromuscular disease associated with a short trinucleotide repeat expansion in exon 1 of the *PABPN1* gene. OPMD (MIM 164300) is rather frequent in Yakut population (~1:11680), that is 1000 times higher as in Caucasoid populations. Patients (n=66) and 134 their healthy near relatives of 44 families were investigated. In all patients the mutation was confirmed by direct sequencing indicating the presence of 14 trinucleotide repeats (GCN), where as healthy individuals bear 10 repeats. Ten SNPs (rs2231301, rs1950252, rs1535094, rs7142474, rs2239579, rs8020117, rs2295126, rs2268330, rs7161120, rs4981469) in OPMD locus were examined. The genotype frequencies obeyed the Hardy-Weinberg equilibrium for all loci in both groups. Three SNPs (rs2231301, rs2239579, rs4981469) revealed strong association with OPMD ($p<10^{-3}$). Ten haplotypes with frequencies from 1.1 to 32.0% were found in control group and seven haplotypes (frequencies range 1.4 - 48.6%) were detected in patients. Patients and controls were characterized by different LD structure in OPMD locus. OPMD mutation is associated with 11 different haplotypes, including 3 major, 2 moderate and 5 rare haplotypes (>13, 6 and <3% frequncies, respectively) indicating possible modest founder effect for OPMD in Yakut population.

P12.187
Mutation analysis of COL1A1 gene in osteogenesis imperfecta patients from Russia

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Osteogenesis imperfecta (OI) is a heritable disorder of connective tissue characterized by wide phenotypic variation mainly including bone fragility. The majority of patients (about 90%) with a clinical diagnosis of OI possess mutations in COL1A1 or COL1A2 genes.
The aim of our study was to identify mutations in COL1A1 gene in OI patients from three Russian populations (Russians, Tatars,Yakuts). We examined 54 patients with OI from 43 families and 50 healthy controls corresponding by age, gender, ethnicity and place of residence. DNA sequencing of 51 exons and exon-intron junctions in COL1A1 gene was performed.
For the first time previously unreported nonsense mutation c.967G>T (p.Gly323X)/1 in COL1A1 gene was identified in heterozygous state in two Russian patients and the novel frameshift mutation c.3541insC (p.Gly1181ArgfsX38) in COL1A1 gene was observed in Yakut patient. We also detected 3 previously described nonsense mutations in 5 Russian patients: c.1081C>T (p.Arg361X), c.1243C>T (p.Arg415X) and c.2869C>T (p.Gln957X), two frameshift mutations in two Tatar patients: c.579delT (p.Gly194ValfsX71) and c.2444delG (p.Gly815AlafsX293) and 1 splicing mutation in one Yakut patient:

c.4005+1G>T. Interestingly, individuals possessing these mutations were characterized by OI type I except for the patient with mutation c.1243C>T (p.Arg415X) clinically diagnosed by OI type 3/4.
The present study revealed two novel mutations in COL1A1 gene in patients with OI type I. Severe OI phenotype was observed in the patient with nonsense mutation c.1243C>T (p.Arg415X), while previously published findings described this mutation in individuals with OI type I.

P12.188
Null *CRTAP* Mutation Associated with Non-lethal OI and Minimal Collagen Deposition in Culture

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Osteogenesis imperfecta (OI), or brittle bone disease, is characterized by bone fragility and deformity. Deficiency of any of the components of the collagen prolyl 3-hydroxylation complex causes recessive OI. The complex contains cartilage-associated protein (CRTAP), prolyl 3-hydroxylase 1 (P3H1) and cyclophilin B; it hydroxylates the α1(I) Pro986 collagen residue. Most *CRTAP* mutations cause a null allele and lethal OI (10/15), while 2 hypomorphic or missense and 3 null alleles were non-lethal. We report a 6 year old Egyptian boy with severe non-lethal type VII OI, who has the length of a 2.5 yr old and DXA z=-3. He is homozygous for a null insertion/deletion *CRTAP* mutation (c.118_133del16insTACCC). He received neridronate since 3 months of age. Similar to other *CRTAP* probands, his dermal fibroblasts synthesize fully overmodified type I collagen (40% OHL/L), which is well secreted into media. Only 5% of proband α1(I)Pro986 is 3-hydroxylated. On real-time RT-PCR, *CRTAP* transcripts are <15% of control. CRTAP protein is absent on Western blotting, with residual P3H1 and normal CyPB levels. Dermal fibril diameters were minimally increased vs an age-matched control, without loose fibril packing. We have noted a severe deficiency (10-15% of control) of collagen deposited in extracellular matrix by proband fibroblasts in long-term cultures, examined by immunofluorescence; the fibrillar network surrounding proband cells was also poorly organized. This data is corroborated by similar deficiency of radioactively-tagged collagen incorporated into matrix in deposition assays. Reduced collagen deposition in matrix is a novel finding which may play a role in the pathophysiology of *CRTAP* mutations.

P12.189
Recessive OI probands in a consanguineous Palestinian pedigree are each homozygous for mutations in a different gene (*FKBP10* and *PPIB*)

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Recessive osteogenesis imperfecta (OI) is caused by defects in genes whose products interact with type I collagen for modification and/or folding. We identified a 5-generation Palestinian pedigree with both moderate and lethal forms of recessive OI caused by homozygous mutations in *FKBP10* or *PPIB*, which encode ER-resident molecular chaperone and isomerase FKPB65 and CyPB, respectively. One pedigree branch includes a child with moderate type XI OI and a homozygous *FKBP10* mutation (c.1271_1272delCCinsA); both parents and one sibling are carriers. *FKBP* transcripts in proband fibroblasts are 10% of control; the mutation is detectable in cDNA from fibroblasts cultured without emetine. FKBP65 protein is reduced in proband cells analyzed by Western blot and immunofluorescence microscopy. Transcripts for other proteins that interact with collagen, CRTAP, P3H1 and HSP47, are increased approximately 2.5 fold in proband cells, reflecting cell stress. Proband collagen gel electrophoresis reveals moderate band broadening, suggesting partial overmodification. Proband collagen α1(I)Pro986 3-hydroxylation is normal, as is collagen T_m. Collagen in proband cells is not aggregated on immunofluorescence. This compares to two cases reported by Alanay et al., in which cells lacking FKBP65 protein had collagen aggregation, while cells with an in-frame *FKBP10* deletion did not. A second pedigree branch includes two children with lethal OI. Both parents carry a 4 nt deletion in exon 5 of *PPIB* (c.563_566delACAG), predicting type IX OI in offspring. Mutant transcripts would likely escape NMD. Persistence of misfolded CypB would be predicted to cause severe dysfunction of the collagen 3-hydroxylation complex, compatible with the lethal outcome.

P12.190
Identification of a novel osteogenesis imperfecta locus in dogs

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Dog genome holds a wealth of information that will benefit human health since most of the canine inherited diseases have similar counterparts in human. We have characterized a novel severe congenital disorder involving multiple skeletal defects among Brazilian Terriers. Affected dogs have clinical symptoms including typical craniofacial features, growth retardation, joint hyperlaxity and osteopenia. Pathohistological analyses revealed abnormalities in bone formation which resemble that of human osteogenesis imperfecta (OI). After exclusion of the known OI genes we performed a genome-wide association study with 7 cases and 11 controls using Illumina 24k arrays and mapped the disease to a autozygous genomic region of 13 Mb in CFA6. The associated region is gene rich and being captured for resequencing to identify the causative mutation for the canine osteodysplasia. The identified locus is a novel OI locus and identification of the gene would provide a candidate for human OIs.

P12.191
The male phenotype in osteopathia striata with cranial sclerosis

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The X-linked skeletal dysplasia Osteopathia Striata with Cranial Sclerosis (OSCS) is caused by truncating mutations in WTX. Females exhibit sclerotic striations on the long bones, cranial sclerosis and craniofacial dysmorphism. Males with OSCS typically have skeletal sclerosis, malformations (omphalocoele, kidney, heart defects), patterning defects and significant pre- and postnatal lethality. Males with milder phenotypes also have WTX mutations. In addition to skeletal sclerosis these individuals can have Hirschsprung disease,

joint contractures, cardiomyopathy and neuromuscular anomalies, but lack congenital malformations. This study seeks to draw genotype-phenotype correlations between these two groups.
WTX is alternatively spliced to encode two protein isoforms (WTXS1, WTXS2). WTXS1 contains binding domains for APC, ß-catenin, WT1 and a region implicated in binding PtdIns(4,5)P2 . WTXS2 excludes the PtdIns(4,5)P2 binding domains and one APC binding domain. Mutations in WTX leading to OSCS are exclusively truncating and cluster according to phenotype; with those producing a severe phenotype located in the 5' region of the gene before the ß-catenin binding domain. Mutations leading to survival but still significant osteosclerosis are, in general, located more 3' in the gene, implying more C-terminal domains also participate in bone accretion. Paradoxically mutations in two mildly affected males predicted to produce a severe phenotype indicate that a correlation between mutation position within WTX and the phenotype severity is not absolute. Characterising new mutations and identifying new phenotypic features help to define functional domains within WTX and provide new information about the cellular pathophysiology of OSCS and the role WTX plays in morphogenesis in general.

P12.192
Autosomal Recessive (ARO) and Autosomal Dominant Osteopetroses (ADO): results from a new UK diagnostic service, focusing on CLCN7-related Osteopetrosis.

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The Osteopetroses are a heterogeneous group of disorders characterised by an increased bone density due to impaired bone resorption.
ARO (OMIM 259700) has a UK incidence of 1:250000. Affected patients may present in early infancy with generalized increase in bone density, predisposition to bone fractures, frontal bossing, progressive deafness/ blindness, hepatosplenomegaly, and severe anaemia. ARO is a heterogeneous disorder currently with 7 associated genes (TCIRG1, RANKL, CA2, CLCN7, OSTM1, PLEKHM1 and TNFRSF11A). Defining the genetic basis for ARO is important in determining whether HSCT therapy may be helpful or contra-indicated.
ADO type II (OMIM 166600) has an incidence of 1:20000 and typically has onset in late childhood or adolescence. Patients may exhibit sclerosis predominantly involving the spine with "sandwich vertebrae", pelvis and skull base. The main complications are fractures, scoliosis and osteomyelitis, particularly affecting the mandible. Hearing/visual loss may affect around 5% of individuals. The gene associated with ADOII is CLCN7.
In July 2010 theThe Bristol Genetics Laboratory launched its new UKGTN sequencing service for 3 of the genes implicated in ARO: CLCN7, RANKL and OSTM1 (using a 48 capillaryABI 3730). Together these genes account for approximately 16%-20% of ARO cases in the UK/European population. In addition, a common mutation in Exon 2 of OSTM1 has been identified in the Middle Eastern ARO population.
We have so far processed 7 patients to date, and found 4 (ADOII) patients positive for sequence changes in CLCN7 (p.Met584Val; p.Gly215Arg; p.Tyr99Cys; p.Arg286Trp). We present the clinical and molecular genetic findings from these positive cases.

P12.193
A patient with Ménierè's Disease is carrier of R822W mutation in *OTOF* gene.

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BACKGROUND: Meniere's disease is a disorder of the inner ear that affects balance and hearing. This condition is characterized by sudden episodes of vertigo, tinnitus, a feeling of pressure or fullness in the ears, and fluctuations in hearing. The cause of Meniere's disease is unknown, although it probably results from a combination of environmental and genetic factors. Otoferlin belongs to a small family of membrane-anchored cytosolic proteins. In the cochlea, otoferlin is believed to play a role in exocytosis of synaptic vesicles at the auditory ribbon synapse of inner hair cells. Otoferlin is encoded by *OTOF* (OMIM: 603681). Mutations in this gene are a cause of neurosensory non-syndromic recessive deafness
CASE REPORT: We reported a case of a 49-year-old male. The first contact with the service of otolaryngology was in 2000. The patient complained of tinnitus in the left ear and vertigo. In 2004, it was detected hearing loss in left ear. In 2005, he began to have bilateral hearing loss. In 2007, last tonal audiometry detected sensorineural hearing loss (60dB) in left ear and mild hearing loss on the right ear and he was diagnosed with Meniere's disease.
RESULTS AND CONCLUSIONS: Patient was heterozygote for R822W mutation (c.2464C>T) in OTOF gene. Role of this mutation in hearing loss is controversial; some authors associate this mutation with non-syndromic deafness, and others authors classify it as a polymorphism without a pathological role. We believe that this mutation may play a role in the developing of Meniere's disease.

P12.194
Digenic inheritance model in Pendred syndrome patients carrying only one disease causing SLC26A4 allele?

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Pendred syndrome is one of the most common causes for autosomal recessive inherited syndromal deafness. It is characterized by sensorineural deafness, enlarged vestibular aqueduct (EVA) with or without cochlear hypoplasia and enlarged thyroid gland. Mutations in the SLC26A4 gene are known to cause syndromic and nonsyndromic hearing loss associated with EVA. Mutations in this gene explain approximately fifty-percent of the cases with hearing loss with EVA. A digenic model for hearing loss with the EVA phenotype has been established. Mutations in either the FOXI1 or the KCNJ10 gene in combination with a pathogenic mutation in the SLC26A4 gene have been reported to cause nonsyndromic hearing loss with EVA.
The aim of our study is to investigate the prevalence of the digenic inheritance in patients with hearing loss with the EVA phenotype, and whether FOXI1 and KCNJ10 genes can be included in the routine diagnostics of Pendred syndrome. We first investigated the FOXI1 gene in a group of patients carrying only one pathogenic mutation in the SLC26A4 gene. In a group of 32 patients only one possible splice site mutation was identified. Investigation of a group of 113 patients without pathogenic mutations in the SLC26A4 gene revealed two possible pathogenic mutations in the FOXI1 gene. The analysis of the KCNJ10 gene in the same patient cohorts is ongoing. Although this study is not yet completed, we can already conclude that yet unknown mutations in regulatory and non-coding regions or other gene(s) are also involved causing hearing loss with EVA phenotype.

P12.195
Periventricular nodular heterotopia and cerebellar hypoplasia: compund heterozygote mutations in a novel gene identified by whole genome sequencing

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Introduction: Next-generation DNA sequencing is empowering scientists to identify genetic variations associated with human disease at higher resolution and greater sensitivity than previously possible. The technology becomes a powerful tool for the diagnosis of genetic diseases and recently, whole exome sequencing was shown to be effective for disease-gene identification and was successfully used to determine the genetic basis of autosomal recessive disorders. Here we present recent data and results from a whole genome sequencing study focusing on a novel syndrome.
Materials and methods: We study two brothers and a sister in a Dutch non-consanguineous pedigree, who suffer from severe delay, epilepsy, microcephaly, and spastic tetraplegia. Dysmorphic facial features and overriding toes were noted. Brain imaging (MRI) revealed in all the

patients diffuse periventricular nodular heterotopia and hypoplasia of the cerebellum. Based on the pedigree an autosomal recessive inheritance was assumed. The genetic cause of this novel syndrome could not be identified using conventional genetic investigations (including candidate gene sequencing, linkage analysis, and expression exon arrays). We analyzed the whole-genome sequences (sequencing performed by Complete Genomics) of six family members and tested multiple inheritance models.

Results: Homozygous mutations in exons were excluded and subsequent analysis of compound heterozygous variants revealed predicted non-conservative changes in promising novel candidate genes of which one is undergoing functional validation.

Conclusion: Using whole genome sequencing we identified the candidate gene for a novel genetic syndrome. We describe the patients' phenotype, the analysis workflow, results and preliminary validation studies.

P12.196
‚Perrault syndrome in a female manifesting carrier of mtDNA 11778G>A mutation'

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Perrault syndrome (PS) is a rare autosomal recessive syndrome, characterized by ovarian dysgenesis, hearing loss and ataxia in female patients. Recently, pathogenic mutations in *HSD17B4* gene have been described in two affected sisters, however, lack of mutation in other families with PS suggests genetic heterogeneity of the syndrome.

We have performed sequencing of 24 coding exons of the *HSD17B4* gene in a 21-years old female with 46,XX karyotype and clinical symptoms of PS: hypergonadotropic hypogonadism, ataxia, intellectual impairment and hearing loss. The open reading frame was free of mutations.

Several patient`s mother`s male relatives are affected by Leber Hereditary Optic Neuropathy (LHON). A test for mtDNA 11778G>A mutation was positive in the affected males and in our patient. Further ophthalmologic assessment revealed advanced optic neuropathy in the patient.

This case is the first reported patient with PS and mtDNA mutation. The authors provide detailed clinical data. Possibility of causal relation between mtDNA mutation and PS and influence of hypogonadism on manifestation of LHON in females are discussed.

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P12.197
The molecular-genetic study of phenylketonuria in Russia

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Phenylketonuria (PKU) is the most common inborn error of amino acid metabolism in Europeans which is caused by a large variety of mutations in the phenylalanine hydroxylase gene (PAH). We analyzed structural features of PAH gene in families with PKU from Bashkortostan Republic (N=124), North Caucasus (N=36) and Kazakhstan (N=20). Consequently we revealed 35 various mutations that spectrum was specific for each region. Our results indicated that R408W mutation accounted for 52% PKU in Bashkortostan and 45% in Kazakhstan. Mutation R261X of the PAH gene is found out only in patients from the North Caucasus with frequency of 38.9% whereas mutation R408W in the given region is revealed with frequency of 13.9%. The frequency of mutation R261Q of the PAH gene was also high in PKU patients from Bashkortostan and Kazakhstan (12.9% and 7.5%, respectively). Other mutations of the PAH gene were identified with frequencies from 3.3% to 0.27% on the average: c.1315+1G>A c.1066-11G>A, R158Q, c.663-664delAG, R252W, P281L, L48S, c.441+5G>T, S349P, I306V, c.47-48delCT, c.168+5G>A, c.208-210del3, R111X, P211T, R243X, A300S, E390G, R413P, Y414C, W169H, c.509+5delG, E280K, Y206X, R243Q, c.1089delG, S350Y, D415N. Four new mutations were revealed as well: R252P, c.116delT, c.1315+del4 in PKU patients from Bashkortostan, F331S - from North Caucasus.83.9% of the studied families with PKU have appeared completely informative for direct DNA-diagnostics, 12.8% - partially-informative, and 3.3% - absolutely not informative.Thus the data of mutational analysis of the PAH gene may be used for prenatal diagnostics and carrier screening in PKU families in studied republics.

P12.198
Identification of three novel single nucleotide changes in the PAH gene observed in Latvian patients with phenylketonuria

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Molecular analysis of 70 Latvian PKU patients has revealed very high genotypic homogeneity, as 73% of the mutant alleles carry the R408W mutation typical for Eastern Europe. 51% (36/70) of patients are homozygous for R408W mutation; the remaining 34 patients are compound heterozygous. Analysis of these patients has revealed 16 different mutations E280K, R158Q, R261Q, P281L, A104D, A403V, E178G, R111X, IVS10-11G>A, IVS12+1G>A, G272X, R261X, E221_D22>Efs, I306V, V230I and three novel single nucleotide changes in the phenylalanine hydroxylase gene: P292T (c.874C>A), K371E (c.1111A>G) and IVS12-1G>A (c.1316-1G>A).

Mutation P292T (c.874C>A) in Exon 8 substitutes proline at residue 292 to thereonine; IVS12-1G>A (c.1316-1G>A) at he boundary of IVS12 and exon 13 affects the conserved dinucleotide at the 3' splice site. Both these mutations were found in association with R408W in patients with severe PKU.

Mutation K371E (c.1111A>G) in Exon 11 replaces lysine at residue 371 with glutamic acid. It was also observed in association with R408W in patient with mild hyperphenylalaninaemia (MHP), who does not need a low-phenylalanine diet. Thereby we suppose that this mutation causes MHP as R408W is a severe mutation with no residual activity of enzyme phenylalanine hydroxylase.

None of the mentioned novel single nucleotide changes had been previously detected in patients' chromosomes and 100 population controls. We assume that these variants are functionally relevant although this need to be confirmed by additional in vitro expression analysis.

P12.199
Neurologic and ocular phenotype in Pitt-Hopkins syndrome and a zebrafish model

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TCF4 haploinsufficiency was recently identified to cause the Pitt-Hopkins syndrome (PTHS), a disorder characterized by severe mental and motor retardation, breathing abnormalities and distinctive facial features. The *Danio rerio* ortholog, *tcf4*, is expressed in several regions in the CNS and the retina during early development. The neurologic and ocular phenotype was characterized in a PTHS-patient with a uniallelic *TCF4* deletion and in a zebrafish model of the disease. While a cerebral MRI-scan in the PTHS-patient at age one year showed a markedly delayed myelination and ventriculomegaly, no structural cerebral anomalies including no evidence for white matter tract alterations were detected on high-resolution MRI with DTI at age 9 years. Structural ocular examinations showed highly myopic eyes and an increase in ocular length, while spectral domain optical coherence tomography (SD-OCT) imaging showed retinal layers and foveal configuration to be normal. Knockdown of *tcf4*-function by injection of morpholino antisense oligos into zebrafish embryos was performed,

and the morphant phenotype was characterized for expression of neural differentiation genes *neurog1*, *ascl1b*, *pax6a*, *zic1*, *atoh1a*, and *atoh2b* resulting in a developmental delay or defects in terminal differentiation of brain and eyes, small eyes with a relative increase in ocular length and an enlargement of the hindbrain ventricle. In summary, *tcf4*-knockdown in zebrafish embryos does not seem to affect early neural patterning and regionalization of the forebrain, but may be involved in later aspects of neurogenesis and differentiation. We provide evidence for a role of TCF4/E2-2 in ocular growth control in PTHS-patients and the zebrafish model.

P12.200
Genetic diagnosis of the Autosomal Dominant Polycystic Kidney Disease by specific sequencing of the PKD1 gene

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Introduction: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is one of the most prevalent inherited disorders, with an incidence of 1:1000. ADPKD is a late-onset multisystem disorder characterized by bilateral renal cysts and cysts in other organs including liver, pancreas and seminal vesicles. Mutations in PKD1 gene (16p13) and PKD2 gene (4q21-q23) are present in 85% and 15%, respectively, of the ADPKD patients. Genetic diagnosis of the PKD1 gene is a tricky task because the presence of, at least, 5 pseudogenes with a 98% similarity with the 5' region of the PKD1 gene.
Objective: To offer an accurate genetic diagnosis to patients with clinical diagnosis of autosomal dominant polycystic kidney disease (ADPKD).
Methodology: Genetic testing of the PKD1 gene in ADPKD patients has been carried out by sequencing throught the 46 exons of the gene. To analyze specifically the 5' region of the PKD1 gene and discard the homologous regions of the known pseudogenes, five specific long PCRs were designed. Obtained fragments were used as templates for the amplification of the first 34 exons of the PKD1 gene by using intronic primers encompassing each exon.
Results and conclusions: This protocol allows obtaining specific sequences for the PKD1 gene excluding homologous regions of the pseudogenes in normal and ADPKD samples.
We can conclude that this validated protocol is a good methodology for genetic diagnosis of ADPKD, avoiding misinterpretation problems produced for the presence of pseudogene sequences. An accurate genetic diagnosis is essential to provide prenatal and preimplantational genetic diagnosis to ADPKD patients.

P12.201**
Altered Hippo signaling in polycystic kidney disease

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is characterized by the formation of many fluid-filled cysts in the kidneys and progressive deterioration of renal function, finally leading to renal failure. Previously we showed that tubular epithelial injury accelerates cyst formation in inducible *Pkd1*-deletion mice. In these mice expression of the Planar Cell Polarity (PCP) component Four-jointed (*Fjx1*) is decreased during epithelial repair but at later stages, in cystic kidneys, *Fjx1* expression is increased.
Additional to a role in PCP-signaling, Four-jointed is also implicated in the Hippo-signaling pathway, which controls organ size by regulating proliferation and apoptosis. In humans, this pathway is also known from its involvement in several types of cancer, and in neurofibromatosis type 2 (NF2). The role of Hippo-signaling, together with the opposing expression pattern of *Fjx1* during epithelial repair and at cystic stages, led us to investigate the activity of the Hippo pathway during these processes.
Examining the Hippo pathway's final effector molecule, the transcriptional co-activator Yes-associated protein (YAP), we found no differential expression between *Pkd1*-deletion mice and controls during tissue repair. However, in epithelia of dilated tubules and cysts, strong nuclear YAP accumulation was observed, which was accompanied by up-regulation of the suspected YAP transcriptional targets *Birc-3*, *Ctgf*, *InhbA* and *Fjx1*. Altered Hippo-pathway activity was confirmed in human renal ADPKD and ARPKD tissues, as well as cystic renal tumors. These data support the notion that during epithelial repair Four-jointed is involved in PCP-signaling, while in cystic kidneys this protein is related to Hippo-signaling and cyst growth.

P12.202
Mutations of PKD1 and PKD2 genes in families with autosomal dominant polycystic kidney disease in Czech Republic

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common form of inherited kidney disease that results in renal failure. ADPKD is a systemic disorder, with cysts and connective tissue abnormalities involving many organs. The disease is caused by mutations of *PKD1* and *PKD2* genes. In the PKD database (http://pkdb.mayo.edu) 846 different variants of the *PKD1* gene and 139 different variants of the *PKD2* gene have been reported.
The direct detection of mutations in both *PKD* genes was performed within research projects in our laboratory. The mutation analysis of *PKD1* gene has so far detected probably causal mutations in 57 families. Only the described nonsense mutation p.R4021X was detected in three nonrelated families and the described missense mutation p.E2771K was detected in two families; other mutations are unique for individual families; 42 mutations are unique for Czech population.
The mutation analysis of *PKD2* gene has so far detected probably causal mutations in 36 families. The frameshifting mutation c.203_204insC was identified in 9 families, nonsense mutation p.Q160X in 6 families. Fourteen mutations are unique for Czech population.
Two families with highly variable clinical course were identified. Trans-heterozygous mutations in both *PKD* genes were found in the patient with end stage renal disease at 28 years. His 65-year-old father with only *PKD2* mutation has mild renal insufficiency and his mother died because of uremia at 45 years. In the second family were found the influence of mosaicism and hypomorphic allele in *PKD1* gene.
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P12.203
Impairment of the tRNA splicing endonuclease subunit 54 (tsen54) gene causes neurological abnormalities and larval death in zebrafish models of Pontocerebellar Hypoplasia

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Pontocerebellar hypoplasia (PCH) represents a group (PCH1-6) of neurodegenerative autosomal recessive disorders characterised by hypoplasia and/or atrophy of the cerebellum, hypoplasia of the ventral pons, progressive microcephaly and variable neocortical atrophy. The majority of PCH2 and PCH4 cases are caused by mutations in the TSEN54 gene; one of four subunits comprising the tRNA-splicing endonuclease (TSEN) complex. We hypothesised that TSEN54 mutations act through a loss of function mechanism. At 8 weeks of gestation, human TSEN54 is expressed ubiquitously in the brain, yet strong expression is seen within the telencephalon and metencephalon. Comparable expression patterns for tsen54 are observed in zebrafish embryos. Morpholino knockdown of tsen54 in zebrafish embryos results in loss of structural definition in the brain. This phenotype was partially rescued by co-injecting the morpholino with human TSEN54 mRNA. A developmental patterning defect was not associated with tsen54 knockdown, however an increase in cell death within the brain was observed, thus bearing resemblance to PCH pathophysiology. Additionally, ENU mutant zebrafish homozygous for a tsen54 premature stop codon mutation die within 9 days post-

fertilisation. To determine whether a common disease pathway exists between TSEN54 and other PCH-related genes, we also monitored the effects of rars2 (PCH1, PCH6) knockdown in zebrafish. Comparable brain phenotypes were observed following inhibition of both genes. These data strongly support the hypothesis that TSEN54 mutations cause PCH through a loss of function mechanism. Also we suggest that a common disease pathway may exist between TSEN54 and RARS2-related PCH, which may involve a tRNA processing related mechanism.

P12.204
An integrated clinical, genetic and epigenetic approach of the Prader Willi syndrome phenotypes in Romanian population

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Prader Willi syndrome (PWS) is a complex disorder whose diagnosis may be difficult to establish on clinical grounds, because of the individual variations in the phenotype and because this one develops with age.

Molecular mechanisms that lead to this disorder could be chromosomal deletions, uniparental disomy (UPD), intragenic mutations and epigenetic modifications in the process of imprinting and rarely reciprocal translocations in 15q11-q13. A common defect is noticed in all cases: loss of parental contribution in this region. This feature coupled with clinical scores and classical karyotyping have been investigated in a group of 36 patients. They were screened for genetic factors, that is: chromosomal translocations (karyotyping), deletions (FISH, fluorescent in situ hybridization) and combined genetic and epigenetic factors: microdeletions and DNA methylation (MSPCR, methylation specific PCR and MS-MLPA, methylation specific multilocus ligation-dependent probe amplification). Among all clinically suspected cases, only 14 cases were confirmed by methylation analyses. The resulted MLPA scheme of microdeletions in the critical region 15q11-q13 in confirmed cases suggested the following mechanism: the imprinting defect can occur secondary to a DNA sequence modification or mutation in a *cis*-acting factor or as primary epigenetic modification in the absence of any DNA sequence change. Primary epigenetic modifications may occur after fertilization and lead to somatic phenotype mosaicism. An indirect *trans*-genetic factor implication, as the *mthfr* gene polymorphism, was investigated in the two families and in the 14 cases. The results of this approach suggested a further link of the PWS with the errors in the parental methylome.

P12.205
Is DMXL1 a Prader-Willi like gene?

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The DMXL1 gene, encoding a WD repeats protein of unknown function, has been proposed recently to be mutated in patients with a Prader-Willi like phenotype (Gokhale et al., abstracts at ASHG 2008 and 2009. Hegde et al, US Patent 2010/0136552 A1), with the finding of heterozygous missense variants in 12/114 patients (10.5%). Eight of these twelve variants occurred in exons 20-24 of this gene. Three of them were cited in the ASHG abstract as de novo mutations but without further confirmation.

We have sequenced either directly (the large exon 24) or after high resolution melting screening (exons 20-23) these exons that encode 20% of the protein, in 328 patients referred to our molecular diagnostic lab for a broadly defined Prader-Willi like phenotype without chromosome 15 anomaly. We have found one already described variant (M1589V) and six novel heterozygous missenses with five of them observed in a single patient each. Three out of these five variants were predicted as possibly or probably pathogenic using Polyphen. Parental samples have been obtained for one case: the R2131S substitution has been found in both affected and healthy relatives and is thus unlikely to be pathogenic. Other parental samples were not available yet.

Among the eighteen variants observed in American and French cohorts, five have since been reported in the dbSNP database and one is also found in an unaffected individual, questioning the implication of DMXL1 in the disease and suggesting a rarer frequency of possibly pathogenic variants than reported initially (2.7% at the most).

P12.206
Prevalence of FMR1 intermediate alleles among women with idiopathic POF in the Basque Country

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Fragile X syndrome is caused by the expansion of a CGG trinucleotide repeat in the 5' untranslated region of the fragile X mental retardation 1 (FMR1) gene. The FMR1 premutation (55-199 repeats) alleles have been associated with premature ovarian failure (POF) and earlier age at menopause. The FMR1 alleles in the intermediate range (45-60 repeats) and in the high end of the normal range (>35) have also been found to be related with POF and early menopause. It has been estimated that approximately 20% of FMR1 premutation women have POF and among females with idiopathic POF, about 3.2% carries the premutation allele. These values are in accordance with our previous results in a sample from the Basque Country (3.5%). To complete the investigation, we have analyzed the prevalence of the intermediate alleles in patients with POF from the Basque Country. We have found that 4,76% of them has an intermediate allele with more than 45 CGG repeats, but this percentage increases to 23,81% when we consider the 35-54 CGG repeats range. In the general Basque population previously analyzed, the percentage of alleles in this range (35-54 CGGs) was 6.8%. The results obtained reinforce the association between the alleles at the high end of the normal range, the intermediate alleles, and the ovarian dysfunction.

P12.207
Identification of a chromosomal locus associated with recessive Primary Ciliary Dyskinesia in a Bedouin family

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Primary ciliary dyskinesia is a rare autosomal recessive genetic disorder, caused by inherited defects of ciliary structure and function. The clinical features reflect the distribution of dysmotile cilia and include neonatal and chronic respiratory distress due to lack of coordinated ciliary movement. In approximately half of PCD patients, there is apparent randomization of left-right axis development, or situs inversus totalis proposed to result from defective function of embryonic nodal cilia.

We have characterized a consanguineous Bedouin family from the Negev, who has two siblings diagnosed with situs inversus and respiratory symptoms and numerous healthy siblings. Linkage to the 13 genes known to be associated with the disease was negated. Further genome wide linkage analysis using the Affymetrix GeneChip mapping 250K array and microsatellite markers was performed.

Homozygosity mapping identified a chromosomal region larger than 16cM. Genotyping the region by analyzing polymorphic markers to all family members has defined a locus of 30Mb on chromosome 18q with a Lod score of 3.0 for multipoint analysis.

Prioritizing genes for search of the mutation and initial sequencing,

was preformed according to the databases of proteome collections, and derived from evolutionarily distant organisms which combines independently assembled ciliary, basal body and centrosome.
Identification of additional genes involved in cilia function will provide new insights into the molecular mechanisms of the cilia and help to develop much needed novel techniques to diagnose subjects with PCD

P12.208
A novel *ABCB11* mutation in an Iranian girl with progressive familial intrahepatic cholestasis

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Progressive familial intrahepatic cholestasis is an autosomal recessive liver disorder originated from biallelic mutation in ABCB11 gene. A 9 year-old girl with cholestasis referred for genetic counseling. The patient was affected by icterus and pruritis from newborn period. She had a family history of cholestasis in two previous expired siblings. Hepatocellular swelling with multi nuclearative canalicular and hepatocellular cholestasis, portal lymphocytic infiltration and fibrosis, newly formed liver nodules, bridging necrosis, bile duct proliferation, giant cells and micro vesicular fat droplet can be seen in proband liver biopsy. Genetic analysis on ABCB11 gene led to identifying of a novel mutation in exon 26. The homozygous mutation was found in sequence 3593 A>G, the mutation lead to a missense mutation at the amino acid level (His1198Arg). This mutation caused PFIC2 due to abnormal function in the bile salt export pump protein (BSEP).

P12.209
Simultaneous loss of heterozygosity and point mutation detection with high resolution melting

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Basal-cell carcinoma (BCC) is the most common type of skin cancer, and shows a continuously increasing incidence. BCCs occur predominantly on head and neck of elderly fair-skinned people, with increased occurrence on the trunk (torso) in recent years. Patched (*PTCH1*) gene is a tumor suppressor gene most often associated with development of BCCs. It is a member of the Hh-Gli signaling pathway, a pathway that plays a major role in embryonic development and stem cell maintenance. Loss of heterozygosity (LOH) of tumor suppressor genes is a frequent event in tumorigenesis, and is most often analyzed by microsatellite typing. Here we offer a fast and efficient method for simultaneous SNP genotyping and mutation scanning, which can also be used for LOH detection. High resolution melting (HRM) analysis is based on differences in melting curves caused by variations in nucleotide sequence during the heating (melting) of the PCR product in the presence of the intercalating dye. Based on our previous experience with HRM, we expanded our mutation screening to LOH analysis with a study of 25 BCC samples. We also compared the LOH results obtained with HRM with those of the conventional microsatellite typing for several STR markers in *PTCH1* region. HRM is an excellent method for both point mutation detection and LOH analysis, with point mutations found in 40%, and LOH in 50% of samples, and the inter-rater agreement between microsatellite and HRM LOH analyses of κ value of 0.869, what can be interpreted as very good agreement between the methods.

P12.210
Gorlin syndrome and PTCH1 gene mutation in two Bulgarian cases

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The nevoid basal cell-carcinoma syndrome (NBCCS), also known as "Gorlin syndrome", is an autosomal dominant disorder of high penetrance, but variable expression. Clinical manifestations are extremely variable and include various skin changes and congenital malformations, as well as predisposition to different neoplasms. Multiple BCCs, odontogenic jaw keratocysts (OKCs), palmar and plantar pits, and falx calcifications are major criteria of the disease.
NBCCS is caused by mutations in the PTCH1 gene
Here we report two patients with NBCCS, caused by frameshift PTCH1mutations. The first patient is a 12-years old girl with craniofacial dysmorphism - macrocephaly, frontal bossing, malformed ears, high arched palate, and multiple nevi. The central nervous system (CNS) manifestations included multiple falx and tentorium calcifications, cavum vergae, cavum septi pellucidi, and occipital arachnoid cysts, mild mental retardation. Family history was positive for Gorlin syndrome (mother and grandmother with multiple BCCs and OKCs; uncle with severe mental retardation, epilepsy, BCCs, OKCs and temporal lobe tumor; an older brother with lethal outcome in early infancy due to congenital hydrocephalus and cerebellar tumor). Molecular-genetic analysis in the proband revealed 2bp deletion in exon 2 of the PTCH1 gene - c.257_258delTC, p.(Leu87Ilefs*1).
The second patient is a 36-years old woman with negative family history. She has been operated for BCCs and OKCs. No CNS or other symptoms were registered so far. The screening for PTCH1 mutations revealed a splice site mutation g.1602+1G>T.
Both established mutations are frameshifting, but they showed different phenotypic expression especially in regard to the CNS manifestations of the disease.

P12.211
The molecular pathology of the inversa subtype of recessive dystrophic epidermolysis bullosa

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All forms of the heritable blistering disorder dystrophic epidermolysis bullosa (DEB) result from mutations in the type VII collagen gene, *COL7A1*. Clinically, DEB is heterogeneous and a spectrum of *COL7A1* mutations has been reported. In spite of paradigms for genotype-phenotype correlation, however, the molecular basis of some rare DEB forms, such as the inversa subtype of recessive DEB (RDEB-I), has not been clearly documented. RDEB-I is characterized by predominant blistering in the body flexures and variable mucosal involvement. To characterize RDEB-I in more detail, we screened *COL7A1* in a cohort of 18 Dutch and UK patients and determined genotype-phenotype correlation. All patients carried homozygous, functionally hemizygous, or compound heterozygous arginine or glycine substitutions within the THD of type VII collagen. However, no clear differences in position of mutated codon or nature of substituting amino acids were evident between these RDEB-I mutations and missense mutations causing other RDEB phenotypes. Our results do not support recent data that have suggested that glycine substitutions close to THD non-collagenous imperfections might underlie RDEB-I, as 7/11 (56%) of mutated glycine codons were sited in the central parts of THD collagenous subdomains. Our data demonstrate that RDEB-I is caused by specific arginine and glycine substitutions within the type VII collagen THD, but the precise pathophysiological mechanism by which they lead to the curious inversa distribution remains obscure. It is plausible that these mutations only lead to disease in body areas

with higher skin temperatures, but functional data to substantiate this are still required.

P12.212
The NIGMS Human Genetic Cell Repository: a resource for biomedical research

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The National Institute of General Medical Sciences (NIGMS) Human Genetic Cell Repository at the Coriell Institute for Medical Research supplies scientists worldwide with high-quality, well-characterized cell lines and DNA for use in biomedical research. Continually expanding, the NIGMS Repository currently offers more than 10,500 different cell lines through its web-based catalog, accessible at ccr.coriell.org. The majority of the Repository is comprised of lymphoblastoid and fibroblast cell lines and DNA from individuals with inherited disorders or chromosomal aberrations, and from apparently healthy individuals representing specific ethnic populations. Induced pluripotent stem cells (iPSCs) are a new addition to the collection: both disease-specific and apparently healthy iPSCs will be available to investigators in 2011. Most of these iPSCs have been reprogrammed from NIGMS Repository fibroblasts. The Repository has also established various differentiated cell lines. In collaboration with the Centers for Disease Control and Prevention's Genetic Testing Reference Material Coordination Program and members of the genetic testing community, genomic DNA reference materials characterized by multiple laboratories using different testing platforms have been developed and are available through the NIGMS Repository. Available reference materials include panels for disorders common in individuals of Ashkenazi Jewish descent, cystic fibrosis, and pharmacogenomic testing. Reference materials for myotonic dystrophy and chromosomal aberrations are now being developed. Genome-wide genotyping of approximately 1,100 samples in the collection has been completed, with data available to researchers through dbGAP. The NIGMS Repository is currently recruiting urea cycle disorder and congenital muscle disease biospecimens through the Urea Cycle Disorders Consortium and Cure CMD, respectively.

P12.213
The p.F110L homozygous mutation of TNNT2 is associated to Restrictive Cardiomyopathy

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BACKGROUND: Pure restrictive cardiomyopathy (RCM) is a very rare disease clinically characterized by impaired relaxation and left ventricular (LV) filling, dilation of both atria and absence of significant LV hypertrophy. Mutations that cause RCM have been identified in several genes that encode contractile proteins like Desmin (DES), Troponin I (TNNT3) and Troponin T (TNNT2). TNNT2 is the tropomyosin-binding subunit of troponin, the thin filament regulatory complex which confers calcium-sensitivity to striated muscle actomyosin ATPase activity. To date no benign mutations of the TNNT2 gene have been reported. Six mutations (p.I79N, p.R92Q, p.R92W, p.A104V, p.D160E and IVS15-1G>A) are characterized by a incidence of sudden death in the setting of hypertrophic cardiomyopathy (HCM). In addition, one family with two affected individuals carriers of the p.F110L mutation has been reported. We here describe the clinical phenotype associated with the mutation p.F110L of theTNNT2 gene within an italian family in which the proband, who showed RCM, was carrying the above mutation in homozygous status.

METHODS: Total DNA was extracted from whole blood. Pre-mutation analysis of the TNNT2 was carried out by means of HRM on the VIIA7 platform. Anomalous melting profiles were subsequent sequenced on the ABI PRISM 3130xl. The proband and relatives underwent clinical and physical workup including cardiac magnetic resonance.

CONCLUSIONS: Althought previously reported, the p.F110L mutation demonstrated to be causatively linked to the RCM phenotype in homozygous status: The carriers of the investigated family did not show any disease sign of the cardiac imaging even in adulthood.

P12.214
Molecular diagnosis of autosomal recessive retinal dystrophies by homozygosity mapping with SNP arrays

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Identity-by-descent **(**IBD) mapping has been instrumental in gene discovery of several autosomal recessive (AR) retinal dystrophies (RDs). The increasing use of genomewide SNP chips makes IBD mapping an accessible tool in a clinical context. We aimed to apply this approach as a molecular diagnostic tool in consanguineous families with several RDs.

In total, 35 patients out of 29 families underwent IBD mapping using 250K SNP chips. Twenty probands were diagnosed with one of the following RDs: Leber Congenital Amaurosis (6), early-onset retinal dystrophy (2), AR retinitis pigmentosa (9) or cone-rod dystrophy (3). In addition, nine probands presented with a syndromic RD: Senior-Loken syndrome (2); LCA with mental retardation (MR) (2) or deafness (1); Usher syndrome type I (1) and II (2) and ARRP in combination with mild MR, growth hormone defect and acromelic dysplasia (1). RD candidate genes located within major IBD regions were screened for mutations by Sanger sequencing. In patients in whom a mutation was found, the clinical record was revisited.

This approach revealed mutations in nine families so far (31%), thereby overcoming the need for laborious gene-to-gene testing of all known disease genes and the need for a well-established phenotype prior to testing. New mutations were found in the following genes: *RDH12* (3), *IQCB1* (3), *USH2A, ABCA4, CNNM4*. In addition, a reappraisal of the clinical diagnosis was made in patients with mutations in *RDH12* and *CNNM4*. Finally, we showed that IBD mapping could also be used in families with a single affected individual.

P12.215
First genetic study of Retinitis Pigmentosa in Italian population by microarray technology.

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Autosomal recessive Retinitis Pigmentosa (AR-RP) displays a high degree of genetic and clinical heterogeneity. The mutations spectrum can vary among different ethnic groups; we report a molecular analysis of Italian AR-RP patients, performed with microarray technology.

94 AP-RP patients originating from different part of Italy were included in the study. Diagnostic criteria were early onset retinal dystrophy with typical pigment clumping, electroretinogram (ERG) severe abnormalities, visual field concentric restriction. All the patients underwent a standard ophthalmic examination. Mutation analysis was performed by a microarray chip APEX (Arrayed Primer Extension), including the most frequent mutations associated with AR-RP (501 mutations in 16 genes). In the cases where the microarray screening detected only one mutation, the whole gene was sequenced. The samples of the patients where the chip could not identify any mutation were analysed for ABCA4 gene with automated sequencing system.

13 sequence variants (in the genes CRB1, USH2A, PDE6A, CERKL, CNGA1, RGR, RDH12, RPE65, MERTK) were identified by means of the microarray technology and other 10 sequence variants could be detected by sequencing. 9 ABCA4 mutations were identified in 8 patients. The protocol allowed the identification of sequence variants out of 34/94 AP-RP patients (36%). USH2A was the most frequently mutated gene. Altogether were characterized 15/94 patients, that were homozygous for one sequence variant in one gene, or heterozygous for two different sequence variants.

Microarray technology may represent a cheap and fast method

for mutation analysis, providing useful information for prognostic evaluation and patients' recruitment for trials of gene therapy.

P12.216
Mutation spectrum of RP1 gene in Saudi Retinitis Pigmentosa patients

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Introduction: The RP1 locus was mapped using linkage analysis in a large autosomal dominant retinitis pigmentosa (RP) family. Mutations in RP1 can cause both autosomal dominant and recessive forms of RP. The RP1 gene encodes a protein of 2156 amino acids and localized in the connecting cilia of photoreceptors.

Method: Familial and single RP cases were enrolled in this study, all came from consanguineous families. DNA samples from all affected individuals were genotyped using the Affymetrix GeneChip Human Mapping 250K Arrays. Genome-wide scan was done using easylinkage software, multiple loci were suggested and evaluation of the potential loci was aided by homozygosity analysis. Direct sequencing of all exons of RP1 was done.

Results: Three novel mutations were identified in RP1 gene in 3 different consanguineous families, and one sporadic case. All mutations identified were clustered in exon 4; two nonsense mutations (p.K1517X, p.W1131X) in families arRP-F028, sRP-19, respectively and one deletion (p.N1143l fs X25) in two unrelated families arRP-F043, arRP-DF01. These mutations were predicted to truncate the RP1 protein by approximately 50% of the full length and make it insensitive to nonsense-mediated decay, leading to the production of truncated proteins which lacks the C terminal.

Discussion:

To date approximately 47 truncation mutations have been identified in RP1, all of which are located on exon 4. Among the truncation mutations only two mutations have been implicated in arRP. Here we are reporting three novel mutations to cause arRP which provides supporting evidence that mutations in RP1 can result in arRP.

P12.217
Mutation analysis of FAM161A in Jewish patients with autosomal recessive Retinitis Pigmentosa and protein localization experiments

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We recently reported the identification of the FAM161A gene as a cause of autosomal recessive retinitis pigmentosa (arRP) in the Israeli population due to null mutations. Aiming to further evaluate the role of FAM161A in arRP, we genotyped the two most common mutations we identified, p.Thr452SerfsX3 and p.Arg523X, and found a prevalence of 11% (21/193) and 3% (5/193) respectively, in a large cohort of Jewish RP patients from Israel and USA. Clinical manifestations of patients with FAM161A mutations varied: the majority had RP phenotype ranging from an early onset (at childhood) to relatively mild sector RP. One of the patients manifested an atypical retinal degeneration.

Due to the high carrier frequency of the p.Thr452SerfsX3 mutation in some Jewish sub-populations (carrier frequency of 1 out of 32 individuals), homozygosity for this mutation was also identified in families with an autosomal pseudo-dominant pattern of inheritance.

To study the localization of the FAM161A protein in the human and mouse retinas, we performed immunohistochemistry and Western Immunoblotting using a commercial antibody as well as custom-made antibodies raised against the C-terminal and an alternatively-spliced exon 4. The analysis in both species revealed a positive and intense signal in the photoreceptors inner-segments as well as a weaker staining in the outer plexiform layer. No expression was evident in the outer-segments. FAM161A mutations are currently the most common cause of arRP in the Jewish population (14%). The function of the encoded protein is currently unknown and additional experiments are needed to clarify its role in the retina.

P12.218
Mutational analysis of RPE65, ABCA4 and RHO genes on Greek patients with retinitis pigmentosa, Leber congenital amaurosis and Stargardt's disease.

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Inherited retinal dystrophies are a phenotypically and genotypically heterogeneous group of diseases that affect more than two million people worldwide. Clinical and genetic heterogeneity have an important impact on genetic testing and counseling of affected families. Typical examples of hereditary retinopathies are Leber congenital amaurosis, retinitis pigmentosa and Stargardt's disease.

In this study we focused our research on the elucidation of the possible role of three genes (*RPE65, ABCA4* and *RHO*) on the pathogenesis of hereditary retinal dystrophies in the Greek population. Mutations in these genes have been associated with several forms of retinal dystrophies. There is no previous study in the Greek population on the correlation between genetic aberrations in the above mentioned genes and clinical phenotype. The first gene that was sequenced was *RPE65* in families with Leber congenital amaurosis and retinitis pigmentosa. Three exons of *ABCA4* in families with Stargardt's disease and retinitis pigmentosa and three exons of *RHO* in families with retinitis pigmentosa, were also sequenced.

Mutation c.272G>A (p.R91Q) in exon 4 of *RPE65* was found in a patient with retinitis pigmentosa and in his healthy mother. This mutation has been associated with retinitis pigmentosa. Another important finding was mutation c.52C>T (p.R18W) in exon 1 of *ABCA4.* This mutation was found in a patient with retinitis pigmentosa. The specific mutation has been associated with autosomal recessive retinitis pigmentosa and Stargardt's disease. We will also discuss additional findings such as polymorphic variants, which were identified in patients as well as in their relatives and possible phenotype/genotype correlations.

P12.219
A hotspot region for LINE1 endonuclease-dependent *de novo* insertion in the *NF1* gene

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RNA-based *NF1* mutation analysis uncovered in 18 index patients splicing alterations, mainly exon skipping events, which were explainable neither by an underlying point-mutation nor by a deletion of genomic sequences. Improved PCR protocols that avoided allelic drop-out of the mutated alleles uncovered *de novo* insertions of 14 *Alu* elements, three LINE1 elements, and one poly-(T) stretch as causes of these splicing defects. All 18 inserted sequences showed the characteristics of LINE1 endonuclease-mediated retrotransposition: the integration sites match the reported LINE1 endonuclease consensus cleavage site; the elements were flanked by target site duplications and contained poly-(A) tails derived from their transcripts. Hence, these 18 pathogenic LINE1 endonuclease-mediated insertions substantially add to the so far ~60 published mutations of this type and represent the largest number of this mutation type characterized in one single human gene. Our findings show that the main effect of retrotransposon insertions within or close to exons is altered splicing. The integration sites were distributed over the entire 282-kb *NF1* gene. However, six different insertions clustered in a 1.5-kb region within the gene. This cluster region contained a specific integration site that was used in two unrelated patients to insert an *Alu*Ya5 element. A second site outside the cluster region was also used twice to insert an *Alu*Ya5 and an *Alu*Yb8 element, respectively. This supports the notion of non-

random insertion of retrotransposons in the human genome. The here identified integration sites may serve to elucidate features that make sequences particularly vulnerable to LINE1 endonuclease-mediated insertions.

P12.220
A Novel MECP2 Gene Mutation in a Tunisian Patient with Rett Syndrome

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Patients with classical Rett show an apparently normal psychomotor development during the first 6-18 months of life. Thereafter, they enter a short period of developmental stagnation followed by a rapid regression in language and motor development. Purposeful hand use is often lost and replaced by repetitive, stereotypic movements. Rett syndrome (RTT) is an X-linked dominant disorder caused frequently by mutations in the methyl-CpG-binding protein 2 gene (MECP2). The aim of this study was to search for mutations in MECP2 gene in two Tunisian patients affected with RTT. The results of mutation analysis revealed mutations in exon 4 of MECP2 gene in the two patients. In one patient we identified a new mutation consisting of a deletion of four bases (c.810-813delAAAG), which led to a frame shift and generated a premature stop codon (p.Lys271Arg fs X15) in transcriptional repression domain-nuclear localization signal (TRD-NLS) domain of MeCP2 protein. With regard to the second patient, a previously described transition (c.916C>T) that changed an arginine to a cysteine residue (p.R306C) in TRD domain of MeCP2 protein was revealed. In conclusion, a new and a known de novo mutation in MECP2 gene were revealed in two Tunisian patients affected with RTT.

P12.221
Mutation analysis of MeCP2 Gene in Egyptian Patients with Rett Syndrome

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This presentation describes molecular analysis of the MeCP2 gene in 15 Egyptian Patients with provetional diagnosis of having Rett Syndrome. The clinical presentations of postnatal microcephaly, regression of milestones, and autistic behaviour with the constant finding of abnormal hand movements (hand washing, flapping, and fingers into mouth) were the main criteria for the clinical geneticist and the neurologist to refere female patients suspected to have Rett syndrome for molecular diagnosis. Direct sequencing analysis of the coding region of MeCP2 gene revield pathogenic mutations in 6 patients out of the 15 ones (40%). Re-examination of mutation negative cases confirming the picture of classical Rett. Conclusion , Large genomic rearrangements or other genes involvement may contribute, to a larger extent than that previously described in other ethnic populations, to the etiology of Rett in Egyptian patients. This is still under further investigations.

P12.222
Study of SLC29A3 gene in Rosai-Dorfman histiocytosis associated with hearing impairment: the French experience.

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SLC29A3 is implicated in a syndromic form of genodermatosis: H syndrome. The major features encounters in H syndrome are Hearing loss, Hyperglycaemia, Heart anomalies, Hypertrichosis, Hyperpigmentation, Hepatomegaly and Hypogonadism. More recently, SLC29A3 mutations have been described in families presenting syndromes associating generalised histiocytosis to systemic progressive features: severe camptodactyly, hearing loss, hypogonadism, hepatomegaly, heart defect and skin hyperpigmentation. We have identified a homozygous novel missense SLC29A3 mutation in a patient presenting with only a progressive sensorineural hearing impairment and a single cervical node. The same mutation was revealed in another patient with Rosai-Dorfman histiocytosis, profound hearing impairment, chronic inflammation with pericarditis and ophthalmological disorders. Both patients originated from North Africa and their mutation may be related to a common ancestor. Our results suggest that SLC29A3 should be studied in patients presenting with histiocytosis and hearing impairment.

P12.223
Severe short-limb dwarfism resembling Grebe chondrodysplasia: report of a third case of the Teebi-Al-Awadi-Opitz-Spranger dysplasia and exclusion of *GDF5* as the causative gene.

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We describe a severe, nonlethal short-limb bone dysplasia in a boy, the first of two twin boys, who was presented to us at age 7 ½ years. An abnormal phenotype was apparent at birth in the first twin with shortening of all limbs. Radiographs showed that while all long bones were affected, the humeri, tibiae and fibulae were particularly short and globoid- or pear-shaped. Moreover, there was marked dysmorphogenesis of the carpal and tarsal bones and phalanges of fingers and toes with delta-shaped phalanges and brachydactyly. No internal organ malformation was detected. His mother suspected deafness since infancy, and hearing loss was confirmed on audiometry; ear CT showed malformation of cochlea and semicircular canals. Sequence analysis of *GDF5* exons gave normal results.
The pattern of clinical and radiographic findings, including hypoacusis as well as the peculiar combination of rhizomelic shortening of the upper limb and mesomelic shortening of the lower limb, are identical to those of two unrelated patients reported in 1986 by Teebi, Al-Awadi, Opitz and Spranger (Hum Genet 74:386-390) . The peculiarity consists in most marked shortening of the rhizomelic segment in the arm and the mesomelic segment in the leg. This third observation seems to confirm this condition as a distinct, specific entity. Absence of mutations in the *GDF5* gene confirms the distinction between this disorder and the Grebe - Hunter-Thompson - DuPan dysplasia family. The molecular basis remains to be determined.

P12.224
Novel mutations in FATP4 associated with Ichthyosis Prematury Syndrome

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Ichthyosis Prematurity Syndrome (IPS) is an autosomal recessive disorder characterized by premature birth, non-scaly ichthyosis and atopic manifestations. The disease was recently shown to be caused by mutations in the gene encoding the fatty acid transport protein 4 (FATP4) and a specific reduction in the incorporation of VLCFA into cellular lipids.
We screened probands from five families segregating IPS for mutations in the FATP4 gene. Four probands were compound heterozygous for four different mutations of which three are novel. Four patients were heterozygous and one patient homozygous for the previously reported non-sense mutation p.C168X (c.504c>a). We identified novel nonsense mutation p.G35X (c.103g>t) in exon 2. Another three sequence variants were predicted to result in missense mutations. p.Q300R (c.899g>a) missense mutation in exon 7 has been reported previously. Missense mutations p.V477D (c.1430t>a) in exon 10 and p.R504H (c.1511g>t) in exon 11 appeared to be novel. All patients had clinical characteristics of IPS and a similar clinical course. All variants were excluded as common variants or polymorphisms by comparing to databases (dbSNP 131) and by the analysis of 200 Scandinavian control chromosomes.

Conclusions: Missense mutations and non-sense mutations in FATP4 are associated with similar clinical features suggesting that missense mutations have a severe impact on FATP4 function. The results broaden the mutational spectrum in FATP4 associated with IPS for molecular diagnosis of and further functional analysis of FATP4.

P12.225
Investigation of association between 5-HTT gene and response to antidepressant drug (Citalopram) in Iranian major depressant patients

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Background: The serotonin transporter (5-HTT) gene is known as the responsible gene for interindividual variation and the genetic susceptibility for psychiatric diseases, such as bipolar mood, schizopherenia and major depression. A common polymorphism in its upstream regulatory region is caused by difference in the number of GC rich, repetitive elements. Deletion or insertion in the 5-HTTLPR creates short (14 repeats), and long allele (16 repeats). Transcription of the L allele is 2 times more than the S allele. Since the serotonin and its transporter are involved in mood control, they can be targeted by antidepressant medications, and specially the selective serotonin reuptake inhibitor: Citalopram.

Materials and methods: In this study the response of 80 patients suffering from major depression disorder, to Citalopram, and its association with their genotypes has been investigated. On the other hand we estimated the allele frequency in Iranian population by analyzing the genotype of 100 normal samples. Our method included PCR followed by running on agarose gel.

Result: frequency of each allele in normal Iranian population is near to 0.5 (L: 0.48 and S: 0.52). 69% of the patients with allele L (L/L or L/S) respond to Citalopram, while just 44% of the S/S patients did not need to change the drug.

Conclusion: our study confirms the hypothesis that the L allele is associated with better response to antidepressant drug (P value=0.033. CI: 95%). But we could not prove any association between the S allele, and risk of major depression (add ratio: 0.923).

P12.226
Laboratory diagnosis of Smith-Lemli-Opitz syndrome in Hungary

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Introduction. Smith-Lemli-Opitz (SLO) syndrome is a severe monogenic autosomal recessive syndrome associated with mental retardation and multiple congenital anomalies. The responsible gene is the 7-sterol reductase (DHCR7). Both life expectancy and quality of life are severely affected by the disease. DHCR7 mutations show a large inter-ethnic variability. Our goal was to set up a diagnostic scheme that includes both biochemical and molecular genetic methodology to diagnose SLO and to detect mutations responsible for SLO in Hungarian patients.

Patients and methods. Ten Hungarian patients were analyzed. Serum 7-DHC and cholesterol levels were measured using an UV spectrophotometry method and using an enzymatic colorimetric method, respectively. For mutation detection, the entire coding region of DHCR7 gene was sequenced.

Results. All patients had elevated 7-DHC level (reference range <0.15 mg/L), ranging from 71.4 to 300.0 mg/L. Cholesterol levels were generally low (between 0.3 and 2.7 mmol/L). The cholesterol/7DHC ratio was abnormal in all cases. 9 patients were compound heterozygous for two causative mutations, while one patient was homozygous for a null allele. One splicing, one nonsense and 8 missense mutations were found. The detected alterations are known to be causative, except the previously unidentified c.374A>G (p.Y125C) which mutation lies in a phylogenetically conservative position. The closest known pathogenic mutations affect amino acid positions 119 and 138.

Conclusions. Using biochemical and molecular genetic methods, the molecular diagnosis of ten Hungarian SLO patients could be established. In addition to the known missense, nonsense and splicing mutations, a novel, most likely pathogenic mutation was identified.

P12.227
Identification of seven new *RAI* mutations in Smith-Magenis syndrome patients without 17p11.2 deletions

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Smith-Magenis syndrome (SMS) is a mental retardation syndrome with sleep disturbance, self injurious behaviours, and dysmorphic features. It is estimated to occur in 1/25 000 births and is associated with interstitial deletions of chromosome 17p11.2 in 90% of cases. RAI mutations are the second main molecular etiology, this gene being located in 17p11.2 in the SMS locus.

We evaluated 33 non-related individuals referred for molecular analysis due to a possible SMS diagnosis, none of these patients carrying any 17p11.2 deletions. Polymerase chain reaction and sequencing strategy identified mutations in *retinoic acid induced 1* gene (*RAI1*) in 7 cases (21%): two non sense mutations (p.Arg80X and p.Gln433X), four heterozygous frame shift mutations leading to protein truncation, and a missense mutation (p.Arg1172Cys). When parental samples were available, we could show that these mutations were *de novo*.

Patients had developmental delay (7/7), facial dysmorphy (7/7) and extremities abnormalities (4/7), major sleep disturbance (6/7) with an inverted rhythm of melatonin release, behavioural problems (6/7) including self aggressiveness, and overweight (6/7). Neither visceral anomalies nor seizures were observed.

All mutations occurred in exon 3 of *RAI1* which codes for more than 98% of the protein, as other mutations previously reported, but there is no hot spot and mutations are scattered all along this third exon.

Several polymorphisms were also observed.

P12.228
The rare parkinsonian phenotype of Machado-Joseph Disease (MJD/SCA3): Analysis of Parkinson's disease associated loci in two unrelated MJD patients

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Machado-Joseph disease (MJD/SCA3) is an autosomal dominant neurodegenerative disorder of late-onset, which is caused by a CAG repeat expansion at exon 10 of the *ATXN3* gene (14q32.1). Clinical heterogeneity observed in MJD, however, is not completely explained by the repeat size. Although Parkinsonism seems to segregate within some MJD families, only a few MJD patients develop parkinsonian features and, therefore, the clinical and genetic aspects of these rare presentations remain poorly understood. The main goal of this work was to investigate genetic variants, previously associated with Parkinson's disease (PD), in two MJD patients of Azorean background, displaying the rare parkinsonian phenotype. Patient 1 is a 40 year-old female (onset at 30 years of age), initially presenting as a pure parkinsonian phenotype (similar to the phenotype previously reported for her mother). Patient 2 is a 38 year-old male (onset at 33 years of age), presenting an ataxic phenotype with parkinsonian features (not seen either in other affected siblings or in his father). Both patients presented an expanded *ATXN3* allele with 72 CAG repeats. Screening of mutations and polymorphisms in PD associated loci, namely *PARK2*, *LRRK2*, *PINK1*, *DJ-1*, *SNCA*, *MAPT*, *APOE*, and mtDNA $tRNA^{Gln}$ T4336C, revealed the absence of PD mutations in the analyzed loci. Both patients, however, presented PD associated polymorphisms in *DJ-1* and *APOE* genes. Additional analysis in larger MJD series would be necessary to better ascertain the putative influence of these loci in the MJD phenotype.

P12.229
A new spastic paraplegia locus/gene (*SPG47*)

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Hereditary spastic paraplegias (HSP) are rare inherited neurological disorders. Our objective was to map the responsible gene in a large consanguineous family of Saudi-Arabian origin excluded for linkage to most autosomal recessive loci. A genome-wide scan was undertaken using 6090 SNP markers covering all chromosomes. The genome-wide search identified a single candidate region on chromosome 4, exceeding the multipoint LOD score threshold of +3 (Zmpt=4.6). Two additional Saudi Arabian families tested with microsatellite markers covering the candidate interval also showed linkage to this new locus (Zmpt=2.4 and +3.0). Patients from the smallest linked family carried the same homozygous haplotype as in the originally mapped kindred and allowed to narrow-down the interval to 3.3 cM containing 24 genes. Mutations in the coding region of 23 candidate genes were excluded and only one missense variant affecting a strongly conserved amino-acid was identified in patients and was absent in >500 Caucasian and North-African controls. Phenotypic presentation in 10 patients was suggestive of a relatively pure and early onset HSP, rarely associated with mental deterioration or thin corpus callosum at cerebral MRI. The mapping of a novel AR-HSP locus further demonstrates the extensive genetic heterogeneity of this condition.

P12.230
Mutations in *REEP1* Cause SPG31 with Alteration of Mitochondrial Functions

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Hereditary spastic paraplegias (HSP) constitute a clinically and genetically heterogeneous group of neurodegenerative disorders characterized at least by slowly progressive spasticity of the lower extremities. Mutations in the *SPG31* gene, *REEP1*, were recently associated with a pure dominant form of the disease. We sequenced all exons of *REEP1* and searched for rearrangements by MLPA in a large panel of 175 unrelated HSP index patients from kindreds with dominant inheritance, with either pure (n = 102) or complicated (n = 73) forms of the disease, after exclusion of other known HSP genes. We identified 12 different heterozygous mutations, 11 of which were novel, in 12 different families, with either a pure or a complex phenotype; three mutations affected the splicing of the gene, two were exon deletions, four were missense mutations and three were small deletions or point mutations introducing premature stop codons. The overall mutation rate in our clinically heterogeneous sample was 4.5% in French families when taking into account HSP cases with other known genetic entities. The phenotype was restricted to pyramidal signs in the lower limbs in most patients but X had a complex phenotype associating cerebellar ataxia, tremor, dementia or a Silver. Interestingly, we evidenced, for the first time, abnormal mitochondrial network organization in fibroblasts of one patient in addition to mitochondrial energy production impairment in his muscle biopsy. Our results indicate that SPG31 is a relatively frequent form of HSP, with variable phenotype and incomplete penetrance associated with altered mitochondrial dynamics and functions.

P12.231
Allele-specific gene expression for evaluation of *SMN* functional efficiency

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Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disorder and a leading genetic cause of infantile mortality. In SMA, the *SMN1 is deleted or destroyed by mutation, while the neighbouring, nearly identical SMN2 fails to generate adequate levels of full-length SMN protein*. *SMN2* copy numbers is inversely correlated with the severity of the SMA phenotype. Total and SMN- FL RNA production was analyzed in peripheral blood of a reduced cohort of 24 subjects: 5 with homozygous deletion of *SMN1*, 11 healthy deletion carriers and 8 individuals from general population carrying 2 copies of *SMN1*, by using a new and highly accurate method that assesses allele-specific gene expression. We used base pair exchanges in exon7 and exon8 to distinguish between transcripts deriving from SMN1/2 genes. *SMN1* and *SMN2* resulted transcriptionally equivalent since observed proportions of total RNA transcripts deriving from the two genes were in line with the respective allelic copy numbers across different genotypes. However, they are not functionally equivalent since the production efficiency of SMN- FL RNA of *SMN1* and *SMN2* varied between 55-90% and 5-17%, respectively. Furthermore, the production of FL RNA is highly correlated (P<0.001) with *SMN1* copy numbers increase, while correlation with *SMN2* copy numbers is heterogeneous supporting that SMN2 alleles are not functionally equivalent. Finally, two asymptomatic subjects bearing homozygous deletion of *SMN1* showed significantly higher levels (P < 0.01) of FL RNA respect to affected individuals.
An accurate evaluation of allele-specific gene expression will significantly improve patients classification, follow-up and basic research .

P12.232
Study of the involvement of the poliovirus receptor (PVR) in childhood spinal muscular atrophy and postpolio syndrome

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by mutations in the SMN1 gene. The highly homologous copy, SMN2, is considered a phenotypic modifier (when more copies, less severe) but is unable to prevent the disease. This correlation is not absolute and patients with the same number of SMN2 copies may manifest different SMA types (I, most severe, II or intermediate, and III the less severe) suggesting possible modifier genes in SMA. Postpolio syndrome (PPS) refers to manifestations that appear many years after acute poliomyelitis infection and occurs in approximately 50% of cases. Despite the clinical course of PPS is relatively slow, PPS can present with multiple symptoms and signs including fatigue and weakness. Given that SMA and poliomyelitis share some characteristics as motor neuron diseases, we proposed to investigate the involvement of the poliovirus receptor (PVR) in 95 healthy individuals, 94 PPS, and 126 patients with different SMA types but the same SMN genotype (homozygous absence of SMN1 and three SMN2 copies). We analysed seven tagSNPs of the PVR gene (rs1042640; rs1058402; rs17714931; rs203709; rs203710; rs3538129; rs714948). In SMA chronic patients, the presence of the T allele of the rs714948 polymorphism was significantly associated with a milder phenotype (type III) (chi square test p=0.041). Furthermore, there were no significant differences in the genotype distribution of the PVR SNPs between SMA or PPS patients and healthy individuals. Further investigations are warranted to unravel a possible effect of the PVR receptor in SMA. Supported by FIS 08-0729.

P12.233
A leaky splicing mutation affecting SMN1 exon 7 inclusion explains an unexpected mild case of spinal muscular atrophy

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder with symptoms usually appearing during early childhood. The disorder is due, in most cases, to homozygous deletions of the *SMN1* gene or, in rare cases, to small hemizygous *SMN1* intragenic mutations. Here we describe the identification and characterization of c.835-3C>T,a novel SMA-causing mutation detected in the intron 6 of the single *SMN1* allele of a type IV SMA patient. This patient carries only 2 copies of *SMN*2, the partially functional *SMN1* paralog. We demonstrate both *ex vivo*, using minigene splicing assays, and *in vivo*, by comparative multiplex RT-PCR analysis of patient RNA, that c.835-3C>T is a deleterious splicing mutation that induces a modest but unequivocal exclusion of exon 7 from the *SMN1* transcripts. We suggest that this effect may be due to the creation of a high-affinity binding site for the splicing repressor protein hnRNP A1 overlapping the splice acceptor site of exon 7. Importantly, the "leakiness" of this splicing mutation explains the exceptionally mild phenotype of an ambulatory 70-year-old SMA patient. Our findings support the current therapeutic strategies aiming at correcting exon 7 splicing in SMA patients, and bring clues about the level of exon 7 inclusion required to achieve a therapeutic effect.

P12.234
Characterising the pro-survival function of the survival of motor neuron (SMN) protein.

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Purpose: Spinal muscular atrophy (SMA), a neurodegenerative disorder primarily affecting motor neurons, is the most common genetic cause of infant death. This incurable disease is caused by the absence of a functional *SMN1* gene which leads to a critical reduction in full length SMN protein. The SMN protein has been linked to numerous cellular functions, including snRNP assembly and survival in motor neurons. However, exactly how SMN protein imparts its cell survival function is still unknown. This study aimed to characterise the pro-survival function of SMN and the relationship between the anti-apoptotic protein Bcl-xL and SMN.

Methods: To assess the relationship between SMN and Bcl-xL *in* vitro, recombinant adenoviral vectors were used to over-express proteins. This relationship was also assessed *in vivo* using a transgenic mouse model over-expressing SMN. The pro-survival mechanisms used by SMN were also assessed in an Akt/PI3 kinase inhibition model. Cell homogenates and mouse tissue were analysed using western blot analysis and real-time RT-PCR, to determine protein and transcript levels respectively.

Results: We found a significant relationship between Bcl-xL and SMN proteins. We found Bcl-xL expression was significantly reduced in SMA patient fibroblasts. Over-expression of SMN could increase Bcl-xL levels by 6 fold, while Bcl-xL over-expression significantly increased SMN protein and transcript levels. Over-expressed SMN protein also protected against Akt/PI3 kinase pathway inhibition, and blocked caspase-3 activation by preventing calpain mediated cleavage of procaspase-3. This study has determined how SMN reduces caspase-3 activation and links the Bcl-xL protein to SMA pathogenesis.

P12.235
Genetic deficiency of tartrate-resistant acid phosphatase is associated with skeletal dysplasia, cerebral calcifications and autoimmunity

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Vertebral and metaphyseal dysplasia, spasticity with cerebral calcifications, and strong predisposition to autoimmune diseases are the hallmarks of the genetic disorder spondyloenchondrodysplasia (SPENCD). We mapped a locus in five consanguineous families to chromosome 19p13 and identified mutations in ACP5, which encodes tartrate-resistant phosphatase (TRAP), in 14 affected individuals and showed that these mutations abolish enzyme function in the serum and cells of affected individuals. Phosphorylated osteopontin, a protein involved in bone reabsorption and in immune regulation, accumulates in serum, urine and cells cultured from TRAP-deficient individuals. Patient-derived dendritic cells exhibit an altered cytokine profile and are more potent than matched control cells in stimulating allogeneic T cell proliferation in mixed lymphocyte reactions. These findings shed new light on the role of osteopontin and its regulation by TRAP in the pathogenesis of common autoimmune disorders. Pharmacologic modulation of osteopontin may thus provide a novel therapeutic angle for diseases like systemic lupus erymathosus and multiple sclerosis.

P12.236**
Tartrate resistant acid phosphatase deficiency causes a bone dysplasia with autoimmunity and a type I interferon expression signature

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Rare Mendelian disorders demonstrating overlap with common diseases are of interest because of the insights they can provide into complex-disease pathogenesis. To this end, we studied ten individuals showing features consistent with the immuno-osseus dysplasia spondyloenchondrodysplasia (SPENCD). Of particular note was the diverse spectrum of autoimmune phenotypes observed in these patients, including systemic lupus erythematosus (SLE), Sjögren's syndrome, haemolytic anaemia and thrombocytopenia. Haplotype data indicated the disease gene to be on chromosome 19p13 and linkage analysis yielded a combined multipoint lod score of 3.6. Sequencing of the *ACP5* gene, encoding tartrate resistant acid phosphatase (TRAP), identified biallelic mutations in each of the patients studied, and *in vivo* testing confirmed a loss of expressed protein. All eight patients assayed demonstrated elevated serum interferon alpha activity.

SLE patients frequently demonstrate an increased expression of type I interferon stimulated genes. To determine if a similar 'interferon signature' was present in SPENCD patients, we undertook whole-genome microarray analysis, followed by confirmatory qPCR. We identified 18 genes which were greater than four-fold over-expressed, 15 of which are recognized as interferon stimulated genes.

Our findings, recently published in Nature Genetics[1], reveal a previously unrecognised link between TRAP activity and interferon metabolism, and highlight the importance of type I interferon in the genesis of autoimmunity.

1. Briggs et al. Tartrate-resistant acid phosphatase deficiency causes a bone dysplasia with autoimmunity and a type I interferon expression signature. Nat Genet. 2011. **43**(2): 127-131.

P12.237
Mutational analysis of the NPHS1, NPHS2, PLCe1 and WT1 genes in Greek children with clinical diagnosis of steroid resistant nephrotic syndrome(SRNS)

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Several genes are implicated in the pathogenesis of autosomal recessive SRNS. The exons and intron/exon boundaries of the genes *NPHS1* (nephrin, 29 exons), NPHS2 (podocin, 8 exons), PLCe1 (phospholipase C epsilon 1, 29/31 exons) and *WT1* (Transcription factor Wilm's Tumour-1, exons 8&9) were directly sequenced in 38 (30 sporadic, 8 familial) SRNS patients (3months-18years). Two congenital NS patients had pathological *NPHS1* genotypes: one had three mutations (p.Cys217fsX in *cis* with p.P264R and *trans* to p.S366R), the other homozygous for p.S366R. Three sporadic SRNS patients had pathogenic *NPHS2* genotypes: p.R138Q homozygous, p.R168H homozygous and p.R229Q/p.A295T compound heterozygous. The novel p.A295T was predicted pathogenic in *silico* (SIFT http://blocks.fhcrc.org/sift/SIFT.html, polyphen http://genetics.bwh.harvard.edu/pph). *PLCe1* gene analysis revealed three homozygotes for the previously described p.R1246X (2 related/familial, 1 sporadic). Six patients carried de-novo *WT1* mutations, including 5 known: one case IVS9+5G>A, one case p.R394Q, one case p.D396N, two cases p.R394W. Mutation p.R366H in the sixth case was novel, predicted pathogenic in silico. Excluding the case with p.D396N who was phenotypically male (XY karyotype), all other cases were phenotypically female, with XX karyotype in four although one p.R394W case had an XY karyotype. Heterozygous mutations were found in several familial cases: 3 with the *NPHS2* gene p.R229Q mutation and 2 with the *NPHS1* gene p.N188I mutation, although no di-genic inheritance was observed. In conclusion 14/38 cases were characterized with pathogenic mutations in *NPHS1, NPHS2, PLCe1* or *WT1,* indicating that molecular investigation of these genes is useful to support definitive diagnosis and management of pediatric SRNS.

P12.238
Functional characterization of mutations in THAP1 causing dystonia 6

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Dystonias represent a group of heterogenous movement disorders, characterized by involuntary twisting, repetitive movements and abnormal postures. Dystonia 6, a monogenic form of primary (isolated) dystonia, is associated with mutations in the *THAP1* gene; however, its molecular pathophysiology is not well understood. THAP1 is a transcription factor with a sequence-specific DNA-binding zinc-finger domain at the N-terminus (THAP-domain) and a putative nuclear localization signal (NLS) towards the C-terminus. Recently, we demonstrated that THAP1 specifically binds to the *TOR1A*-promoter and represses *TOR1A*-expression. The *TOR1A* gene is involved in primary dystonia 1.

By screening a large cohort of 700 patients with dystonia, we identified several new mutations located within the THAP-domain as well as mutations resulting in truncated THAP1 proteins. To investigate the functional consequence of the missense mutations, we performed luciferase-reporter gene-assays demonstrating that missense mutations within the THAP-domain result in a decreased or almost abolished THAP1-mediated repression of *TOR1A* expression. In contrast, amino acid exchanges within the C-terminal region of THAP1 did not influence its activity.

Secondly, we analyzed the intracellular localization of GFP-tagged wildtype THAP1, THAP1 with truncating mutations, and a missense mutation within the NLS (I149T) via confocal laserscanning microscopy. Mutations in the NLS, its complete or partial loss, resulted in an impaired transport of THAP1 protein into the nucleus.

In summary, our data provide evidence that missense mutations in the DNA-binding THAP1-domain as well as mutations leading to a partial or complete loss of the NLS sequence both result in a disturbed transcription factor activity of THAP1.

P12.239
Mutations in the 5'UTR of *ANKRD26,* the Ankirin Repeat Domain 26 Gene, Cause an Autosomal-Dominant Form of Inherited Thrombocytopenia, THC2

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THC2, an autosomal-dominant thrombocytopenia described so far in only two families, has been ascribed to mutations in MASTL or ACBD5. We found that ANKRD26, another gene within the THC2 locus, and neither MASTL nor ACBD5, is mutated in eight unrelated families. ANKRD26 was also found to be mutated in the family previously reported to have an ACBD5 mutation.We identified six different ANKRD26 mutations, which were clustered in a highly conserved 19 bp sequence located in the 5' untranslated region. Mutations
were not detected in 500 controls and are absent from the 1000 Genomes database. Available data from an animal model and Dr. Watson's genome give evidence against haploinsufficiency as the pathogenetic mechanism for ANKRD26-mediated thrombocytopenia. The luciferase reporter assay suggests that these 5'UTR mutations might enhance ANKRD26 expression. ANKRD26 is the ancestor of a family of primate-specific genes termed POTE, which have been recently identified as a family of proapoptotic proteins. Dysregulation of apoptosis might therefore be the pathogenetic mechanism, as demonstrated for another thrombocytopenia, THC4. Further investigation is needed to provide evidence supporting this hypothesis.

P12.240
Identification of novel TAAD loci on 2q37 and 7p15 in a large Dutch family: further evidence of disease heterogeneity and support of a bi-or multigenetic inheritance model

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Background: It is estimated that 20% of the non-syndromic thoracic aortic aneurysm and dissection (TAAD) cases is inherited in an autosomal dominant pattern with variable expression and reduced penetrance. With mutation analysis of the currently known TAAD genes; *TGFBR1*, *TGFBR2*, *ACTA2* and *MYH11*, less than 20% of the familial TAAD cases are solved, indicating that there are more TAAD genes to be found.

Methods and results: We have characterized a large Dutch family in which TAAD was inherited in a seemingly autosomal dominant inheritance pattern, with reduced penetrance and variable expression. No segregating alterations in the known TAAD genes were identified by routine diagnostic testing. Genome-wide linkage analysis followed by fine-mapping exposed two novel TAAD loci on chromosome 2q37.1 and chromosome 7p15.3-7p14.3. The coding sequence of all protein-coding genes within these loci was analysed using a targeted massively parallel sequencing approach, by two independent exon-centric enrichment methods. However, the causal mutation was not identified.

Conclusions:

The identification of two novel TAAD-loci within one family that do

not contain any of the known TAAD genes and do not overlap with formerly reported loci, emphasizes the heterogeneity of the disease and suggests an interaction of two or more genetic factors in the development of TAAD within this family. In general, this assumption would explain the reduced penetrance and variable expression in familial TAAD. In the reported family, causative alterations may be present in non-coding regulatory sequences.

P12.241
Myelination delay in MCT8 mutated patients: role of MCT8 and MCT10 in thyroid hormone transport in human oligodendrocytes

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We have identified mutations in the Monocarboxylate Transporter 8 (MCT8) gene, which encodes a thyroid hormone (TH) transporter, in patients initially presenting a Pelizaeus-Merzbacher-Like Disease (PMLD) phenotype related to an early diffuse hypomyelination. In contrast with classical PMLD patients, MRI follow-up of those patients demonstrated an improvement of the white matter signal suggesting a severe delay in myelination rather than a permanent myelin defect. TH are known to play a major role during the central nervous system (CNS) development not only in neurons but also in oligodendrocytes, the myelinating cells of the CNS. Nevertheless, the way TH enters those cells remains unidentified. Different TH transporters have been described expressed in the CNS but only at the blood-brain barrier in endothelial cells (OATP1C1, MCT8) and in neurons (MCT8, LAT1/2). Another newly identified transporter, MCT10 would be expressed in CNS white matter.
The myelination delay observed in MCT8 patients suggests that MCT8 may play a role in oligodendrocytes maturation. Using the human MO3.13 oligodendrocyte cell line, we found that (1) in proliferating cells, both MCT8 and MCT10 are expressed; (2) MCT8 and MCT10 each ensure 40% of T3 transport, suggesting that at least a third transporter may be implicated; (3) during MO3.13 early differentiation, MCT8 and MCT10 are differentially regulated, MCT8 becoming the predominant transporter. Those results demonstrate for the first time that MCT8 and MCT10 are implicated in TH uptake in oligodendrocytes at least in proliferative progenitors and during early differentiation, when TH have been demonstrated promoting oligodendrocyte differentiation.

P12.242
Mutational analysis of the TRPC6 gene in adult patients with steroid-resistant idiopathic nephrotic syndrome

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Focal segmental glomerulosclerosis (FSGS) and minimal change disease (MCD) are frequent cause of nephrotic syndrome (NS). TRPC6 mutations are the cause of idiopatic NS in about 2-7 % of steroid-resistant patients. Eleven different TRPC6 gene mutations were identified. These mutations lead to a late onset kidney disease and a variable rate of progression to end stage renal disease. The aim of the study was the identification of mutations/polymorphisms in adult patients with steroid-resistant FSGS/MCD.
Patients and methods: 40 patients (22 females, 18 males) with steroid- resistant FSGS/MCD were studied. The mean age of the onset of NS was 39±20.7 years.200 healthy Czech individuals formed control group with mean age 58.4±19.5 years. High resolution melting method (HRM) was established for all 13 exons of the TRPC6 gene. Suspected samples were analysed by direct sequencing on ABI Prism 3130 Genetic Analyzer.
Results: No TRPC6 gene mutation was identified. Two polymorphisms were described: in exon 1 C43T (P15S) with prevalence 32.5% of heterozygotes and in exon 4 C1211T (A404V) with prevalence 20% of heterozygotes (resp. 1% of T/T homozygotes). The prevalence of heterozygotes in control group was only 16.5% for C43T and 20.5% for C1211T (resp. 2.5% of T/T homozygotes). T allele of C43T polymorphism in exon 1 was significantly more frequent in patients with FSGS.
Conclusions: TRPC6 gene mutations are a rare cause of FSGS/MCD in adult patients in Czech Republic. The C43T polymorphism could have some influence on FSGS.
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P12.243
Different genetic alterations cause similar clinical symptoms of contiguous TSC2-PKD1 gene syndrome.

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Tuberous sclerosis complex (TSC) is a genetically determined disorder with an estimated incidence of 1 in 6000 live births. The main features of TSC are benign tumors called hamarthomas frequently found in brain, skin and kidney. Convulsions, seizures and mental retardation are also common features of the entity.
A TSC2-PKD1 contiguous gene syndrome caused by complete or partial TSC2 and PKD1 genes has been reported in patients with typical TSC and polycystic kidney disease symptoms. TSC2 is located adjacent to PKD1, the gene for autosomal dominant polycystic kidney disease (ADPKD), in tail to tail orientation. Large deletions in TSC2 are relatively common (5.6%) and substantial fraction of them (50%) extends into PKD1 gene.
In five TSC patients with renal cysts we performed analysis for large mutations using commercial and home-made sets of Multiple Ligation-dependent Probe Amplification (MLPA) probes for TSC2-PKD1 genes and direct sequencing for TSC1-TSC2 genes. Cytogenetic and FISH assays were performed on metaphases derived from a culture of peripheral blood lymphocytes.
Two of five patients are carriers of large multi-exon deletions. A heterozygous deletion in TSC2 from exon 1-15 was found in a third patient but no visible alteration in PKD1. Another patient demonstrated a partial deletion in the joining region between TSC2 and PKD1 that was only detectable by FISH analysis. The last patient we examined was negative for all mutational screening.
Here we describe several patients with manifestation of TSC and ADPKD, suggesting contiguous gene syndrome but with substantial differences in their genetic alterations.

P12.244
Mutational analysis in TSC1 and TSC2 genes in patients with tuberous sclerosis complex

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Tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterized by the development of multiple hamartomas in many organs, with an incidence of 1 in 6,000 to 1 in 10,000 live births. TSC is caused by mutations in tumor suppressor genes TSC1 and TSC2. Mutations in TSC2 are about five times more common than mutations in TSC1 in sporadic cases, whereas the ratio is 1:1 in large families with multiple generations affected. Mutations in TSC1 and TSC2 are very heterogeneous and no hotspots have been reported, making the molecular diagnosis of the disease extremely difficult. Here, we have studied eight patients from the Republic of Macedonia with a clinical diagnosis of TSC and their parents. The methodology included MLPA analysis for the detection of gross gene rearrangements and sequencing analysis of all exons and exon/intron boundaries of the TSC1 and TSC2 genes. We have detected five mutations, of which four in TSC2 and one in TSC1 gene. The TSC2 defects included

two gross deletions (deletion of exon 1 and upstream sequence and deletion of exons 1-15), one small deletion (c.4318delC) and one missense mutation (c.772A->T). The TSC1 mutation was small deletion (c.1431_1434del|AGAA). All mutations were *de novo* events and have been previously reported. The mutational spectrum among our patients suggests that the best approach for detection of mutations in TSC patients would be MLPA analysis of TSC genes for the detection of gross rearrangements, followed by sequencing analysis for the detection of point mutations.

P12.245***

Complete exon sequencing of Usher syndrome genes as a diagnostic tool in a therapeutic perspective

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Usher syndrome (USH) combines sensorineural deafness with blindness. It is inherited in an autosomal recessive mode. Early diagnosis is critical for adapted educational and therapeutic choices, and for genetic counseling. To date, nine causative genes have been identified for the three clinical subtypes (USH1, USH2 and USH3). Current diagnostic strategies make use of a genotyping microarray that is based on the previously reported mutations. The purpose of this study was to design a more accurate molecular diagnosis tool.

We sequenced the 366 coding exons and flanking regions of the nine known USH genes, in 54 USH patients (27 USH1, 21 USH2 and 6 USH3). **Results:** Presumably pathogenic mutations were detected in 48 patients (89%). In addition to biallelic mutations in one of the USH genes, presumably pathogenic mutations in another USH gene were detected in seven patients (13%), and another patient carried monoallelic mutations in three different genes. Notably, none of the USH3 patients carried detectable mutations in the only known USH3 gene, whereas they all carried mutations in USH2 genes. Most importantly, the currently used microarray would have detected only 30 of the 81 different mutations that we found, of which 39 (48%) were novel.

Based on these results, complete exon sequencing of the currently known USH genes stands as a definite improvement for molecular diagnosis of this disease.

P12.246

Ophthalmic status of patients with Usher syndrome

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Usher syndrome (USH) is a relatively rare genetically heterogeneous autosomal recessive disorder with three clinical subtypes (USH I, II, III). It is due to some mutations in any of 10 genes (MYO7A, CDH23, PCDH15, SANS, USH1C, USH1G, USH2A, GPR98, DFNB31, CLRN1). The frequency of USH was estimated to be 1/12,500 (Germany), 1/23,000 (USA), 1/28,000 (Norway). Here we reported on 24 patients with USH aged from 6 to 38 yr. Nobody had clinical signs of vestibular dysfunction. Severe sensorineural deafness symptoms of the III-IV degrees were observed in all patients in their first year of life. Ophthalmic status included decrement in visual acuity, reduction of the peripheral visual field, defective dark adaptation, nyctalopia, disturbance of colour vision in their 4th- 5th year of life. Typical key findings of fundus disturbance for retinitis pigmentosa (RP) including bone spicules were found. Among children and youth aged from 5 to 25 years RP signs were clearly diagnosed as the disease process of the 1-II degrees. This stage was characterized by satisfactory visual acuity (0.7-0.5) and uninjured visual fields which did not result in patient disabling. Patients aged older than 30 yr had RP of the III-IV degrees with symptoms of the secondary optic atrophy including severe abnormality of visual fields down to its disappearance, visual acuity decreasing down to photoperception and loss of colour perception. Complex ophthalmic investigation and trophic medication(antioxidative, neuroprotective medicines, vitamin-mineral compex) were regularly applied.

P12.247

Intracellular localization of the Type 1 VWD candidate VWF gene variants

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Von Willebrand Factor (VWF) is a multimeric glycoprotein that maintains hemostasis in the vascular system. VWF is secreted mainly from endothelial cells either constitutively or stored for regulated release in Weibel Palade Bodies (WPB). Partial quantitative deficiency of VWF is defined as Type I von Willebrand disease (VWD). Clinical diagnosis of Type I VWD is complicated due to incomplete penetrance and variable expressivity of VWF. Deficiency in the biosynthestic pathway or increased clearance of plasma VWF might be responsible for the decreased VWF levels. The purpose of the study is to explore the effect of four VWF mutations (p.M771I, p.L881R, p.P1413L, p.Q1475X), that were reported as candidate mutations in Type I VWD patients on the intracellular localization of VWF in a heterologous cell system. p.M771 and p.L881 and p.P1413 are located in D', D3 and A1 domains, respectively, implicated in multimerization and WPB storage. p.Q1475 is located in A2 domain. The candidate mutations were generated on VWF cDNA expression vector by in vitro site directed mutagenesis. Intracellular localization of the variants were analyzed in the transiently transfected HEK293 cells by immunoflourescence antibody staining and confocal scanning laser microscopy. Diffuse staining was observed for the recombinant p.M771I and p.Q1475X VWF variants. However, similar intracellular pseudo-WPB storage was observed as wild type recombinant VWF for the VWF variants having p.L881R and p.P1413L changes. This study demonstrated that while p.M771I and p.Q1475X mutations impair intracellular localization of the recombinant VWF, p.L881R and p.1413L mutations did not affect the pseudo-WPB storage in HEK293 cells.

P12.248

Screening of *SOX10* and *MITF* regulatory regions in Waardenburg patients

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Waardenburg syndrome (WS) is a rare auditory-pigmentary disorder that exhibits varying combinations of sensorineural hearing loss and pigmentation defects. Four subtypes are clinically defined based on the presence or absence of additional symptoms. WS2, which is defined by the absence of additional features, results from mutations within the *SOX10* and *MITF* genes. However, 70% of WS2 remain unexplained, suggesting that other genes could be involved and/or that mutations within the known genes escape screening performed so far. The crucial role of SOX10 and MITF during melanocyte development and the identification of several of their regulatory regions prompted us to search for mutations within some of these regions in 30 WS2 unexplained cases. In case of *MITF*, we tested the involvement of the well known melanocyte-specific (M) promoter region and a distal regulatory region (MDE) located 14.5 kb upstream from exon 1M. In case of *SOX10*, sequence conservation analysis led to the identification of at least 5 segments directing expression in overlapping populations of neural crest derivatives. Previous functional studies revealed the role of at least two of them (U1 and U3) during melanocyte development. No deletion was identified upon QMF-PCR analysis of the *MITF* promoter and the 5 *SOX10* regulatory regions. So far, sequencing of U1, U3 and the 2 *MITF* regions led to

the identification of 2 variations not previously reported in dbSNP: 1 in close proximity to the MDE sequence, and 1 within U1. Their functional consequences should be tested in the near future.

P12.249***

Identification and functional analysis of *SOX10* missense mutations in different types of Waardenburg syndrome

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Waardenburg syndrome (WS) is an auditory-pigmentary disorder that exhibits varying combinations of sensorineural hearing loss and pigmentation defects. Four subtypes are clinically defined based on the presence or absence of additional symptoms. In particular, the absence of additional features characterizes WS2, whereas the association with Hirschsprung disease defines WS4. WS is genetically heterogeneous with six genes already involved, including *SOX10*. Since 1998, about 50 heterozygous mutations or deletions of this gene have been described in patients presenting with WS2 or WS4, with or without myelination defects of the peripheral and central nervous system (PCWH). The mutations characterized so far are mostly truncating mutations, removing part of the protein. Only 3 missense mutations were described. Here, we report the identification of new SOX10 missense mutations in 11 patients, associated with a variety of phenotypes ranging from WS2 to PCWH, and describe the functional consequences of each of these mutations on the main SOX10 characteristics and functions. Altogether, our functional assays highlight deleterious effects of all the mutations tested. Some mutants present with partial cytoplasmic and/or subnuclear redistribution, some lose their DNA binding and/or their transactivation capacities on various tissue specific target genes. Intriguingly, several mutants were redistributed in nuclear foci. To our knowledge, such redistribution was not described so far during functional studies of patients' mutations identified in other SOX genes. Whether it is a cause or a consequence of the mutant pathogenicity remains to determine, but it could help identify new SOX10 function and mode of action.

P12.250

Screening of ADAMTS10, FBN1 and ADAMTS17 in a cohort of 17 patients with Weill Marchesani syndrome

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Weill-Marchesani syndrome (WMS) is a rare disorder characterized by short stature, short and stubby hands and feet, stiff joints and dislocation of microspherophakic lens which causes severe myopia, glaucoma or cataract. Despite clinical homogeneity, two modes of inheritance have been reported: autosomal dominant and autosomal recessive. We first showed that Fibrillin 1 (FBN1) is responsible for the dominant form of WMS. We identified an in frame deletion of 24 nucleotides within a LTBP motif (exon 41, 5074_5097del) of FBN1. In the mean time, we identified three distinct mutations in ADAMTS10 (A Disintegrin-like And Metalloproteinase domain (reprolysin type) with ThromboSpondin type 1 repeats) responsible for the autosomal recessive form of WMS.

Following this initial study, we have collected the samples of 17 additional WMS families. We identified ADAMTS10 mutations in 2/17 patients comprising 3 novel mutations (2 missense and one nonsense mutations) and FBN1 mutations in 5/17 including 5 novel missense mutations. The screening of ADAMTS10 is still in progress for 3 cases and the screening of FBN1 is still in progress for 5 cases. Recently, three homozygous mutations in ADAMTS17 have been identified in 3 WMS cases. We therefore decided to screen ADAMTS17 in 2 cases unlinked to ADAMTS10 and FBN1. Finally we analysed the microfibrils of fibroblasts of WMS patients by EM and showed no major changes in their microfibrillar structure.

Our clinical and genetic findings suggest that ADAMTS10, ADAMTS17 and FBN1 play a critical role in crystalline lens and connective tissue formations.

P12.251

c.3402delC (p.Ala1135GlnfsX13) is the most frequent mutation among Venezuelan Wilson disease patients.

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Wilson disease is an infrequent autosomal recessive disorder of copper transport, characterized by accumulation of intracellular copper, with consequent severe hepatic and neurological disturbances; the gene, *ATP7B*, encodes an ATPasa Cu(2+)-transporting beta polypeptide. To date, more than 380 mutations have been reported in many different populations, but a few ones show high frequencies in some areas; the most prevalent mutation in Europe is H1069Q, with frequencies over 65%; in Sardinia, c.-441_-427del accounts for 61.5% of mutated chromosomes; mutation M645R is present in 55% of patients in Spain, and mutation R778L is very frequent in Asians. Deletion c.3402delC at exon 15 has been reported in worldwide populations with very low frequencies (usually below 2%), and is not present in Asians; the only exception are Brazilian patients, with a mutation frequency of 31%. In Venezuela, DNA analysis in 21 independent Wilson disease families gathered during the last 24 years revealed 10 different mutations in the *ATP7B* gene; 80% of patients with detected mutations were compound heterozygotes, but mutation c.3402delC had a frequency of 21.4% and a very wide distribution in the country. Haplotype analyses using 5 intragenic single nucleotide polymorphisms (SNPs) apparently did not support a single ancestral origin for the mutation, despite all remote ancestors of the families came from Venezuela. Either a high mutation rate or gene conversion both in Brazil and Venezuela might suggest an Amerindian origin for it, but neither SNPs frequencies nor the specific Amerindian marker D9S1120*275 in Venezuelan carriers seem to support this hypothesis.

P12.252

Molecular basis of Tricho-Hepato-Enteric syndrome: characterization of mutations in *TTC37*

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The Tricho-Hepato-Enteric (THE) syndrome is an autosomal recessive condition marked by early and intractable diarrhea, hair abnormalities, IUGR, facial dysmorphy and immune defects. Mutations in *TTC37* which encodes the putative protein Thespin, have recently been associated with THE syndrome. Here, we extend the pattern of *TTC37* mutations by the description of 13 novel mutations in 10 patients

with a typical THE syndrome and report their potential effects on transcription. Different types of mutation were observed: frameshift mutations splice site altering mutations or missense mutations. Most of them are predicted to create a premature stop codon or to alter splicing, including missense mutations. The effects of splice sites mutations and missense on sequence transcripts were analysed by direct sequencing of RNA transcripts from lymphoblastoid cells, for 5 mutations. The tested samples exhibited abnormal sequences due to the modification of splicing. In particular, we observed skipping of exon 23 and exon 25, leading to transcripts and possibly deleted proteins, suggesting that these exons are encoding important functional regions in the protein. Quantitative expression was investigated on transcripts lacking exon 25 from lymphoblastoid cells and from hair root cells and showed that levels of expression were not significantly reduced as compared to heterozygous or normal controls. This sustained the hypothesis that the deleted proteins are expressed but are functionally altered.
Further mutational effect studies will lead surely to better characterize the localisation and functional properties of Thespin.

P12.253
Identification of a novel canine chondrodysplasia mutation

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Skeletal dysplasias are a heterogeneous group of disorders affecting the bone and cartilage tissues. Chondrodysplasias are characterized by disturbed endochondral ossification which often results in disproportionate short stature or dwarfism. We report here the identification of a recessive canine chondrodysplasia mutation in a gene that represents a new candidate for human chondrodysplasias. The novel mutation was identified in Norwegian Elkhounds which have been previously described to suffer from a generalized disturbance in the endochondral ossification process. Our pedigree analysis suggested a recessive mode of inheritance for the disorder. We genotyped nine cases and nine controls using Illumina's canine SNP chip of 22K SNPs to map the disease locus. Genome-wide association analysis revealed a 2 Mb disease associated locus on CFA17. Mutation screening of a plausible causative gene revealed a homozygous nonsense mutation in all affected dogs, predicted to result in a premature stop codon in the encoded protein. Segregation of the mutation was studied further in 157 unrelated population controls none of which were homozygous for the mutant allele. A genetic test has been developed and is offered to the breed to help identify carrier dogs. Furthermore, we have initiated screens in human patients with related phenotypes. We hope our findings will improve the understanding of the endochondral ossification processes and related molecular pathways in health and disease.

J12.01
Detection of polymorphisms in the mannose-binding lectin gene and their influence on the phenotype in Slovak patients with cystic fibrosis

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Cystic fibrosis (CF) is the most common lethal inherited disease among Caucasians. The cause of death in 90% of the CF patients is respiratory insufficiency due to chronic inflammation caused by bacteria (Pseudomonas aeruginosa, Burkholderia cepacia).
Mannose-binding lectin (MBL) protein is an important mediator component of the innate immune defense system. It has been shown that MBL variant alleles causing low MBL serum levels are associated with an increased risk of different types of infections. In exon 1 of the MBL2 gene, three single nucleotide polymorphisms (nucleotide substitutions) cause independently low serum levels of MBL at codon 52 (arginine with cysteine, allele D), at codon 54 (glycine with aspartic acid, allele B) or at codon 57 (glycine with glutamic acid, allele C). Y/X promoter polymorphism in codon -221 has a significant effect on the MBL serum levels.
Polymorphisms in exon 1 and promoter region were typed by single base primer extension assay (SNaPshot) in 91 Slovak patients with cystic fibrosis and 100 healthy controls. Concentrations of MBL protein were determined in 34 patients by a Sandwich enzyme-linked immunosorbent assay. Spirometric and microbiological data were collected from medical records.
No association was found for the MBL2 structural variants and severity of cystic fibrosis lung disease. But significant association was observed between MBL2 variants and protein levels and positive implications between MBL2 genotypes, immunoglobulin G and atopy. These findings provide correlation between MBL2 genotypes and protein level, but variant alleles are not associated with poor diagnosis in Slovak CF patients.

J12.02
A novel heterozygous mutation in a Spanish family with Best's vitelliform macular dystrophy

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Best's vitelliform macular dystrophy (BVMD, OMIM#153700) is a dominantly inherited, juvenile onset form of macular degeneration with variable expresivity, which is characterized by abnormal accumulation of deposits of lipofuscin-like material in the subretinal space and a reduced Arden ratio of the electro-oculogram (EOG).
Mutations in the gene VMD2 are associated with BVMD. The gene VMD2 is expressed in the plasma membrane of the retinal pigment epithelium and encodes the besthrophin-1 protein. Besthrophin-1 is a transmembrane protein with underdetermined function although it has been proposed to act as a Cl− channel activated by intracellular Ca2+ and/or as a channel regulator.
We report a non consanguineous Spanish family with BVMD. Family members were examined ophtalmologically to assess their phenotype. After exclusion of the known mutations using the Vitelliform Macular Dystrophy (BEST-VMD) mutation array (Asper Biotech), direct sequencing of the eleven exons of VMD2 was performed to find possible mutations.
The affected sisters were heterozygotes for a novel VMD2 gene missense mutation p.I73L, (c.217A>C) in exon 3 and the father and paternal grandmother were heterozygous for the same mutation. This mutation was no present in their clinically unaffected mother. The father and the eldest sister presented typical lesions of Best's disease. The paternal grandmother presented central areolar atrophy consistent with the late stage of Best's disease.
In conclusion, a novel heterozygous mutation in VMD2 gene causative for Best's disease was found, proving that mutational screening is a useful tool in the molecular diagnostic of bestrinopathies.

J12.03
Characterization of a novel mutation in the GJB2 gene in congenital sensorineural hearing impairment

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The hearing impairment is the most frequent sensory defect worldwide. More than half of children have a genetic origin and correspond to non-syndromic hearing loss (NSHL). Autosomal recessive is one of the most frequent pattern of inherited hearing impairment. Mutations in the GJB2 gene, encoding the conexin 26 gap-junction protein, are the principal cause of NSHL. Currently, more than 100 different recessive mutations have been found in the GJB2 gene; 35delG is a hot-spot in the white population, 235delC in East Asian and 167delT in the Ashkenazi Jewish. In the present study we ascertained 20 unrelated Mexican families with NSHL with an autosomal recessive inheritance

and identified the novel homozygous GTG/GCG mutation (V63A) in the *GJB2* gene. We also found a high prevalence of heterozygous. In this work we report a novel mutation in the GJB2 gene in NSHL.

J12.04
Genotype-phenotype correlation in compound heterozygote patient with severe salt wasting form of CAH

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Eleven common pseudogene-derived mutations in the CYP21A2 gene, encoding the 21-hydroxylase enzyme, account for approximately 95% of all mutations causing congenital adrenal hyperplasia(CAH).
Here we present a patient with a salt wasting (SW) form of the disease. As a newborn he presented with a severe clinical symptoms: vomiting, failure to thrive, complete virilisation. Biochemical work up showed low sodium, high potassium, and elevated 17OHProgesterone level (>75 nmol/L) confirming CAH. Karyotype was 46.XX. Uretrocistography confirmed male urethra with small uterus and sinus urogenitalis. No testicular tissue was seen on ultrasound. We performed direct molecular detection of eleven CYP21A2 mutations by allele created restriction sight method (ACRS).
Our patient was compound heterozygote for three severe mutations: IVS2, Q318X, R356W and one mild V281L mutation in the CYP21A2 gene. Although the compound heterozygotes for different CYP21A2 mutations usually have a phenotype compatible with the presence of the milder gene defect, SW clinical presentation was probably a result of the influence of the other three severe mutations. His mother was homozygote for V281L and Q318W and heterozygote for R356W, without any signs or symptoms of the disease, while the father was heterozygote for IVS2. Interestingly, the brother harbored none of the tested mutations. It is likely that these complex findings probably resulted from small or large gene conversions, multiple mutations events or peculiar recombination events due to the tandem-repeat organization of the gene cluster.
The genotype-phenotype correlation observed in the patient and his mother suggest that the genotype cannot be completely predictive of phenotype.

J12.05
Molecular genetic characteristics of spinal muscular atrophies in Belarus

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Proximal spinal muscular atrophies (SMAs) are autosomal recessive diseases characterized by degeneration of the anterior horn cells of the spinal cord, resulting in symmetrical limb muscle atrophy and weakness. Carrier frequency is 1 in 40-60. Critical region involved in pathogenesis of SMA is 5q11.2-q13.3.
We performed analysis of common mutations of two main genes - SMN1 and NAIP. The presence of exons 7 and 8 of the SMN1 gene was determined using PCR and restriction enzyme digestion. For detection of gene NAIP deletion exons 4, 5 and 12 were amplified using triplex PCR.
Group of 59 probands was included in this study. Deletions was detected in 39 probands: both exons 7 and 8 of SMN1 gene and exons 4 and 5 of NAIP gene - 10 patients (26%), exons 7 and 8 of SMN1 gene - 23 (59%), exon 7 only of SMN1 gene - 5 (13%) and exons 4 and 5 only of NAIP gene - 1 (3%). Carrier status was established using MLPA method. All parents were heterozygous carriers of same mutation that was indentified in kids. Prenatal DNA-diagnosis of SMA was performed in six cases. Four fetuses were found homozygous carriers of mutations.

J12.06
Analysis of 3'-UTR region polymorphic variant +3379A/T of human angiopoietin-1 (Ang1) in sickle-cell disease (SCD) patients.

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SCD is a genetic disorder relatively common among Afro-descendants and caused by a missense mutation in the beta polypeptide chain of hemoglobin. SCD shows distinct clinical evolutions in different patients. Vasoocclusion induced by irregularly shaped sickle cells and subsequent endothelial activation may play the central role in the pathophysiological process. Ang1 regulates blood vessel development, remodeling, and maturation. Recently Ang1/Ang2/Tie-2 system has emerged as a non-redundant regulator of endothelial activation. The T/T genotype of the polymorphic variant +3379A/T in the 3∋-UTR region of human Ang1 was reported as associated with stroke risk reduction in Chinese Han population. As stroke is one of the major complications of SCD, we designed the present study to find out allele and genotype frequencies of this variant in a group of SCD patients from Brazil, with the objective of investigating its possible involvement in stroke risk.
We analyzed 92 SCD patients (S/S and S/C hemoglobin genotypes). The allele frequencies of the +3379A/T variant were: 0.71 of A allele, 0.29 of T allele. HWE test showed marginally significant deviation from equilibrium: p=0.044 (Pearson's goodness-of-fit chi-square, df=1), p=0.034 (likelihood ratio chi-square, df=1), p=0.06 (Exact test).
The observed allelic frequency of A allele is different from that previously reported for CEPH (0.47) and YRI (0.23; low coverage panel) populations of the HapMap project. As the next step, we plan to increase sample size of SCD patients with and without stroke incidents and include control individuals in order to evaluate the association of stroke risk with different genotypes.

J12.07
The role of SNPs in 5'HS4 beta globin LCR in modifying the thalassemia phenotype in Iranian patients

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5'HS4 core region of beta globin LCR was analysed in 100 Iranian individuals with normal hematological indices and 100 patients with beta^0 mutation in both alleles. Coinheritance of three SNP alleles in 5'HS4 was observed in a normal individual in heterozygous state. We have recently observed and reported the same coinheritance in a carrier individual with unexpected thalassemia intermedia phenotype. Despite it's presence in the normal population, the role of this rare polymorphic pattern in altering the clinical picture of our reported patient cannot be ruled out as it may only exert its influence when at least one beta globin allele is affected. Furthermore, There was a statistically significant difference in the frequency of G allele in the polymorphic palyndromic TGGGG(A/G)CCCCA region of 5'HS4 between the normal population and thalassemia patients with two affected alleles. This suggests a linkage disequilibrium between the G allele in the palyndromic region of 5'HS4 and thalassemia mutations.

J12.08
New case of inherited Congenital Central Hypoventilation Syndrome in a family of the young girl with fatal central apnea

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Congenital Central Hypoventilation syndrome (CCHS (MIM 209880)) is a rare disorder, characterised by absence of adequate autonomic control of respiration with decreased sensitivity to hypercapnia and hypoxia. Children with CCHS have normal ventilation while awake but persistent alveolar hypoventilation and/or apnea during sleep.
The paired-like homeobox gene (PHOX2B) was identifed as the gene

underlying CCHS, with a heterozygous expansion of +5 to +13 alanines in a 20 alanines stretch being present in about 90% of patients. Other mutations are found in a minority of cases.
Here, we report the results of mutation screening in a family having a young girl with fatal central apnea. DNA test revealed that the proband is a heterozygous carrier of +6 alanine expansion in the PHOX2B gene. Expansions of that size are always resulted in severe clinical symptoms with continuous ventilatory dependence.
More than 90% of PHOX2B expansions occur *de novo* in CCHS probands, with only 5-10% of unaffected parents showing somatic mosaicism for the gene defect seen in child. Analyses performed for both parents of the affected girl identified the same size expansion in asymptomatic father which means the rare case of parental transmission of the disease. Carrier father presented a mutant peak of lower intensity (35%) than the wild-type, which is highly suggestive of somatic mosaicism explaining his asymptomatic status.
This is the first case of CCHS analysis in Belarus, showing germinal expansion leading to central apnea and ventilatory phenotype in proband, and somatic mosaicism without any clinical symptoms in her father.

J12.09
Double heterozygosity in GJB2 heterozygots of autosomal recessive non-syndromic hearing impairment: assessment of GJB6 gene

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Hereditary non-syndromic hearing loss is inherited in autosomal recessive pattern in about 80% of cases. Up to 50 percent of all autosomal recessive nonsyndromic hearing impairment cases in different populations are caused by mutations in the GJB2 gene which encodes the gap-junction (GJ) protein connexin (Cx) 26. However 10 to 50 percent of patients with GJB2 mutations have only one mutant allele. GJB6 encoding Cx30 is expressed in the same inner-ear structures as GJB2, with which are normally co-assemble into GJs in the cochlea. This study aims to determine whether variations in GJB6 can be the second mutant allele causing the disease in the GJB2 heterozygous cases studied.
We examined 44 unrelated GJB2 heterozygous autosomal recessive non-syndromic hearing loss subjects for any sequence variations in GJB6, using polymerase chain reaction followed by direct sequencing. Sequence analysis showed a heterozygous substitution of c.173C>T in GJB6 in one of the cases which leads to the change of p.Pro58Leu in amino acid sequence.
So this allelic variation in GJB6 can be considered as the second mutant allele in patients with only one GJB2 mutant allele. Further examinations on control cases and co-segregational family studies are in progress to confirm the detected variation as the second mutation responsible for the disease.

J12.10
The prevalence of SCN5A mutation in Iranian Brugada Syndrome patients

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Background. Brugada Syndrome (BrS) is an autosomal dominant disorder characterized by right bundle branch block (RBBB) and ST-elevations in V1-V3, apparently normal heart and high risk of sudden cardiac death (SCD). The *SCN5A* gene encodes the main cardiac sodium channel. Mutations of this gene were detected in 10%-30% of patients with BrS.
Material and Methods. Clinical investigations: Iranian patients with BrS and or familial history of SCD were examined by standard ECG, 24-hour HM, and procainamide challenge test (PCT). Genetic analysis: All coding exons of *SCN5A* gene were sequenced.
Results. Forty unrelated probands with BrS were studied. Mean age was 39 y.o., and M:F ratio was 13:1. Family history of SCD was 45.2%, and acute ventricular events were registered in 73.8% probands. Half of cohort had spontaneous Brugada type-1 ECG, the rest became positive on PCT.
Twenty-two probands were fully screened for SCN5A mutations. Sequencing of the rest is in progress now. We found 3 *SCN5A* mutations: A735V, Y1434X, and del_KPQ1505-1507 (14% of probands with complete screening). Familial analysis of delKPQ carriers reveals some patients with LQTS and BrS both.
Conclusion. In this study we detected *SCN5A* mutations in 14% of BrS probands with completly sequenced gene of interest. The mutation rate in Iranian BrS cohort is in accordance with data published worldwide. Deletion KPQ can produce different phenotype, and exact mechanism of clinical expression is to be clarified. Further molecular investigations are required to elucidate genetic background of BrS in Iran.

J12.11
Screening of LRTOMT gene mutations (DFNB63 locus) in Iranian patients with autosomal recessive nonsyndromic hearing loss

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Background: Prelingual hearing loss occurs in 1 in 1000 newborns and is inherited in more than 60% of cases. Approximately more than 80% of inherited cases are nonsyndromic. Nonsyndromic hearing loss is so heterogene and more than 100 loci are known to be related to it.
Objectives: To determine LRTOMT gene mutation profile and frequency in Iranian patients.
Methods: 114 patients with nonsyndromic hearing loss from Hormozgan province in south of Iran were recruited for this study. DNA was extracted using Phenol-Chloroform method. The screening of the LRTOMT gene in exons 3,5 and 9 was performed using PCR and direct sequencing.
Results: These three sequenced exons (3 , 5 and 9) were studied using Chromas software and no mutation was detected in these three exons, in the samples studied.
Conclusions: Screening of 3 exons (3,5 and 9) of LRTOMT gene revealed no mutations, however we recommend to study the whole gene coding region in more samples.

J12.12
Mutational analysis of MFN 2 in hereditary motor and sensory neuropathy patients from Bashkortostan Republic (Russia)

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Hereditary motor and sensory neuropathy (HMSN) is a clinically and genetically heterogeneous disorder of peripheral nervous system. The HMSN frequency in Bashkortostan Republic (BR) is 10,3:100000. We examined HMSN patients from BR and detected spectrum and frequency of specific mutations in mitofusin 2 (*MFN2*) gene - cause the axonal subtype of CMTIIA. The MFN2 gene (1p36.22) codes a protein mitofusin 2 - an important factor in the fusion of mitochondria. Molecular-genetic investigation of HMSN in 165 unrelated families showed 9 different nucleotide changes in the *MFN2* gene. Four of them haven't been described previously. Four nucleotide changes are supposed to be disease causing mutations: c.776G>A (p.Arg259His) (0,6% among all HMSN types in total of patients sample) and c.2113 G>A (p.Val705 Ile) (1,2%) - previously described mutations, c.775C>T (p.Arg259Cys) (0,6% in total sample of patients, 2,1% - among patients of Tatar ethnic origin) and c.2171T>C (p.Ley724Pro) (0,6% in total sample, 2,1% - among Bashkirs) - new mutations, not detected among healthy family members and controls (n=80). Five revealed nucleotide changes appeared to be gene polymorphic variants:

c.892G>A (p.Gly298Arg), c.957C>T (p.Gly319Gly) and c.1039-222t>c - described polymorphisms, c.175+28c>t and c.2204+15t>c - new nucleotide changes in gene introns. It is found that the contribution of HMSN IIA type to general structure of HMSN among patients from BR is 5,1%. The received data will contribute to optimize medical and genetic consulting of HMSN families in our region.

J12.13
Molecular study of Leber's hereditary optic neuropathy in Brazilian patients

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Leber Hereditary Optic Neuropathy (LHON) is a mitochondrial disease characterized by sudden loss of vision in both eyes, due to optic nerve degeneration. Currently, 17 main LHON associated mutations were published, three of which account for 95% of the cases (primary mutations) and fourteen different mutations account for only 5% of the total (secondary mutations). There is no official data about the frequency of LHON mutations in Brazilian patients. Screening of LHON mutations is important for confirmation of clinical diagnosis, provide prognostic information and more appropriate genetic counseling. Therefore, the aim of this study was to define the LHON mutations frequency in Brazilian patients. We evaluated 55 patients with LHON diagnosis or acquired optic neuropathy of unknown origin. Primary mutations (G11778A, T14484C and G3460A) and secondary mutations were screened. Primary mutations were screened by the method of enzyme restriction and secondary mutations by direct sequencing. Primary mutations were found in 19 patients. The frequencies found were 67% for the G11778A mutation (13 cases), 28% for the T14484C mutation (5 cases) and 5% for the G3460A mutation (only 1 case). The G11778A mutation was more frequent, as well as in others parts of the world. However, the frequency of T14484C mutation was higher while the G3460A mutation frequency was lower. No secondary mutation was found. The absence of these mutations can be attributed to the presence of mutations in regions not analyzed in this study. Analyze molecular allowed us to confirm LHON diagnosis in 38% patients studied.

J12.14
Novel coding variations in C20ORF54 in an Iranian BVVL patient

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Brown-Vialetto-Von Lear (BVVL) is a rare progressive neurological disorder typically characterized by pontobulbar palsy and associate with bilateral nerve failures usually involving the seventh and ninth to twelfth cranial nerves. Fifty eight cases have been reported in the literature. Clinical symptoms often begin with weakness and muscular atrophy and progress to mental and movement disorders. In last phases of disease, patients are unable to do daily activities and they suffer from respiratory and swallowing problems. BVVL is more common in females, with a sex ratio 1:3. However, clinical symptoms are more sever and more rapidly progressive in males. The age at onset of clinical presentations is variable, sometimes even between affected members of the same family. Heredity pattern in multi-case families is variable. Although most families exhibit autosomal recessive inheritance, X-linked and autosomal dominant inheritance with variable expression have also been suggested. In 2010, the C20ORF54 gene was identified to be the BVVL causing gene in all patients examined by autozygosity mapping; mutations were recessive. The protein product of C20ORF54 may be a membrane transporter for riboflavin in the small intestine. Here, we report identification of a new BVVL affected individual born to consanguineous parents. Mutation screening of C20ORF54 gene identified two novel coding variations.

J12.15
Partial duplication of the *PMP22* gene in a patient with hereditary neuropathy with liability to pressure palsies

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INTRODUCTION: Hereditary neuropathy with liability to pressure palsies (HNPP) is an autosomal-dominant disease. Motor and sensory nerve dysfunction due to compression at common entrapment sites is the characteristic clinical presentation. Electrophysiological findings show a distinctive background polyneuropathy, independent of superimposed entrapment neuropathy. It is characterized by diffuse sensory nerve conduction velocity (NCV) slowing. Spite of distal motor latencies are prolonged, there is a minor reduction of motor nerve conduction velocities. Up to 84% of HNPP are due to the 1.5 Mb deletion at 17p11.2-12 containing the *PMP22* gene. This gene codifies for the membrane glycoprotein, Peripheral Myelin Protein 22.
PATIENT: A previously healthy 31-years-old male presented with dropped food due to left peroneal neuropathy with clinical improvement after 3 months. At 17-years-old he presented an episode of dropped wrist. Electrophisiological studies revealed the typical HNPP findings.
METHODS: We performed the genetic testing of the CMT1A/HNPP region using allelotyping studies and MLPA technique.
RESULTS: An exon 3 duplication of *PMP22* gene instead of the common deletion was found.
DISCUSSION: The HNPP phenotype presented in this patient with a partial duplication could be explained because of a frameshift change resulting in a loss of function of the new truncated protein
CONCLUSION: HNNP is a genetically heterogeneous disease and can be due to partial duplications in *PMP22* gene

J12.16
Low-density lipoprotein receptor mRNA level in freshly isolated human familial hypercholesterolemia leukocytes does not correlate with plasma serum cholesterol level

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Aims. Familial hypercholesterolemia (FH) is a common (1:500) monogenic disease caused by low density lipoporotein (LDL) catabolism lowering due to dysfunction of the specific LDL receptor (LDLR). We aimed to investigate if the LDLR mRNA level in freshly isolated peripheral venous blood leukocytes was inversely correlated with plasma level cholesterol in patients with heterozygous FH.
Methods. Total RNA was prepared from 1-2 millions of freshly isolated viable white blood cells and approximately 100 ng of cDNA was used in real-time PCR TaqMan mRNA quantitation test. LDLR gene expression was normalized against ubiquitine C gene expression and plotted versus blood plasma total cholesterol figures.
Results. We found LDLR mRNA level to be highly variable in FH blood leukocytes ranging from 0.2 to 1.8 times content of LDLR mRNA in the control individual. We found that most possible mechanism of LDLR gene dysfunction in common deltaG197 (also known as G218del or FH-Lithuania mutation) variant heterozygous leukocytes is due to mRNA level lowering and not to defects in the intracellular protein maturation and transport. Patients taking up high dose of statins usually demonstrate the elevated level of LDLR mRNA.
Conclusions. We found no inverse correlation between LDLR mRNA level in peripheral blood FH leukocytes and plasma cholesterol level. So far, quantitation of LDLR mRNA in leukocytes cannot be used for prediction of disease severity and efficacy of hypocholestorolemic drug treatment in heterozygous FH.

J12.17
Detection of two *CFTR* large rearrangements in Russian CF patients

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To date more than 1800 mutations have been identified in the *CFTR* gene [http:www.genet.sickkids.on.ca].They involve coding, splicing

and regulatory regions of the gene. To date 48 large rearrangements in *CFTR* gene are found, some of them involves deletions spanning several introns and exons
Two new large rearrangements were found in Russian CF patients.
In family A a routine detection of *CFTR* mutations revealed that an affected child had a homozygous F508del genotype, a father had a heterozygous F508del genotype, whereas a mother didn't have F508del mutation. Intragenic *CFTR* markers analysis showed that an affected child "had lost" maternal alleles of polymorphic markers IVS6aGATT, IVS8CA, M470V (intron 6a, intron 8, exon 10), therewith he had the two alleles at locus IVS1CA, IVS17bCA (intron 1, intron 17b) inherited from the both of parents.
In family B neither of *CFTR* mutant alleles was identified. During intragenic *CFTR* markers analysis it turned out that an affected child failed to have paternal alleles at polymorphic loci IVS1CA, IVS6aGATT, IVS8CA, M470V, but he had two alleles at locus IVS17bCA in intron 17b inherited from the both of parents. Analysis of STR markers at loci D13S141 and DFNB1 on chromosome 13 proved genetic relationship within the family.
Segregation analysis of intragenic *CFTR* polymorphic markers in CF families revealed that deletion of maternal *CFTR* gene overlapped a region from intron 6a to exon 10 at least, and deletion of paternal *CFTR* gene embraced an area from intron 1 to exon 10.

J12.18
First reported splice site mutation (c. 1935+3A>C) of the FGD1 gene in a patient with Aarskog-Scott syndrome

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Introduction: Mutations in the FGD1 gene have been shown to cause Aarskog-Scott syndrome (AAS) (OMIM 305400), an X-linked congenital disorder characterized by distinctive genital and skeletal abnormalities with a broad spectrum of clinical variability. To date, 29 distinct mutations, including a complete deletion, have been reported, and only one of them is a splice site mutation. We present the clinical data of this Spanish patient.
Clinical case: Male, born at term, birth weight 3,050 g, length 50 cm, OFC 34 cm. Unremarkable family history. Referred for clinical investigation because of a delay in global growth and dysmorphic features: frontal bossing, widow´s peak, hypertelorism, large and depressed nasal bridge, short nose, midfacial hypoplasia, long philtrum, brachydactyly and shawl scrotum. At 13 months-old weight 8.595 kg (3rd centile), height 72.5 cm (6th centile), OFC 45.5 cm (19th centile). Patent ductus arteriosus without hemodinamic effects. At 5 years, 4 months-old weight 16.8 kg (14th centile), height 103 cm (5th centile), OFC 49 cm (3rd centile), bone age 3 years-old. Normal/border line developmental outcome. Mutational analysis of the FGD1 gene: c. 1935+3A>C. His mother is carrier of the mutation. This mutation alters the donor splice site of the eleventh intron, possibly resulting in an improperly spliced mRNA.
Summary: We present the first example of an aberrant splicing mechanism of FGD1 as the cause of AAS. The clinical phenotype of the patient is indistinguishable from other patients supporting the evidence of no genotype-phenotype correlation derived from comparison of patients with different mechanisms of mutation.

J12.19
Study of mutation spectra in patients with familial hypercholesterolemia from two major cities in North-West Russia: St. Petersburg and Petrozavodsk

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Introduction: Familial hypercholesterolemia (FH) is a common monogenic disease leading to premature coronary and cerebral atherosclerosis. FH is caused by mutations in the low density lipoprotein receptor (LDLR) gene or in the APOB gene coding for the LDLR major ligand -apolipoprotein B-100. Aim of the present study was to compare spectra of mutations leading to FH development in patients from two major cities in North-West Russia: St. Petersburg and Petrozavodsk.
Methods: We used venous blood of 60 Petrozavodsk FH patients to extract DNA and different methods of molecular biology including PCR, SSCP-analysis, molecular cloning in Escherichia coli, and DNA sequencing. Data were compared with mutation spectra in 100 St.Petersburg FH patients.
Results: 1. R3500Q APOB mutation that is frequent variant in European populations does not occur in FH patients from both cities.
2. None of FH patients from Petrozavodsk carried mutation FH-North Karelia in the LDLR gene, that is typical for the residents of eastern Finland but found only once in St. Petersburg.
3. C139G and G197del LDLR gene mutations that are recurrent in St. Petersburg FH do not occur in Petrozavodsk sample.
4. We have identified new mutation c.192del10/ins8 in two unrelated patients from Petrozavodsk. This mutation represents a complex rearrangement in the 5 'part of the LDLR gene third exon.
Conclusion: New mutation c.192del10/ins8 results in shift of the reading frame and creates a premature stop codon in the LDLR gene fourth exon. Therefore, this mutation is a likely cause of FH in both heterozygous patients.

J12.20
GJB2 caused hearing loss in Armenia is infrequent

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Mutations in Connexin 26 gene (*GJB2*) are responsible for more than half of all cases of prelingual nonsyndromic recessive deafness (PNSRD) in Caucasians. The carrier frequency of the c.35delG mutation in *GJB2* gene was found to be as high as 1-4% in the European populations. The aim of this study was to estimate *GJB2* caused cases fraction and mutation spectrum in 70 Armenian patients with PNSRD. Three pathologic genotype were detected are c.[35delG]+[35delG] in 10 patients, c.[35delG]+[-3202+1G>A, also known as IVS1+1G→A] in 6 patients and [c.35delG]+[p.Glu120del] in one patient. Therefore, the portion of *GJB2* caused deafness is to be 24%. The c.35delG and IVS+1G>A mutations frequencies were most high and were found to be 79% and 18% in all chromosomes with mutations.

J12.21
Neonatal respiratory failure in Saudi's full-term newborn infants associated with *ABCA3* gene mutations

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Neonatal Respiratory Distress Syndrome (RDS) is commonly occurs in preterm babies and may also seen in term babies with Surfactant protein B or protein C deficiency. ABCA3 (ATP-Binding Cassette, Subfamily A, Member 3) maintains pulmonary surfactant homeostasis through transport of phospholipids and proteins to the lung alveolar surface. Recently, ABCA3 mutation was described in pediatric interstitial lung disease patients, including those with chronic pneumonitis of infancy, desquamative instititial pneumonitis and non-specific interstitial pneumontitis. ABCA3 mutation is typically inherited in an autosomal recessive pattern. The incidence and prevalence of neonatal RDS due to ABCA3 mutations are unknown worldwide. In Saudi Arabia, consanguinity were common within the population community and we anticipated that in RDS related to ABCA3 mutations were under diagnosed due to the lack of ABCA3 studies. Here, in this study we investigate and report the ABCA3 mutations findings in seven full-term newborn babies diagnosed with RDS in our institute. Genomic DNA was extracted from patients' blood and sequenced bi-directionaly for all ABCA3 exons using ABI 3130xl Genomic Analyzer. We identified

novel nonsense and missense mutations in our patients that not described previously. Molecular characterization will help in offering families having such disorders a chance to do prenatal/preimplentation genetic diagnosis.

J12.22
The significance of detailed molecular analysis in prenatal diagnosis of Congenital Adrenal Hyperplasia

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A case of prenatal diagnostics in high risk family of congenital adrenal hyperplasia (CAH) is presented. Formerly the detection of two mutations in CYP21A2 gene was diagnosed as CAH. However it is not always correct. It is necessary to take into account the number of copies of the gene that is under consideration.

In the examined family index case was a boy with a neonatal simple virilizing form of the disease with the splice mutation of intron 2 (CYP21A2 gene) in homozygous. The farther had a second normal allele (not inherited by the patient) carrying the Q318X Dup variant (inherited by the fetus). Real-time PCR method was used to determine the copy number variation of CYP21A2 gene. The severe point mutation Q318X was detected in "normal" chromosome with a duplicated gene. The other fetal allele carried the splice mutation of intron 2 of CYP21A2 gene inherited from the mother. So, the fetus had two mutations, but 3 copy of CYP21A2 gene. Thus our final conclusion was the fetus would be healthy.

The correct definition of the Q318X variant as a severe mutation of a single functional gene or a gene-duplicated nondeficient allele is very important for genetic counseling and prenatal diagnosis.

J12.23
LMNA-associated congenital muscular dystrophy in Russian families

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Mutations in *LMNA* gene cause a variety of diseases affecting striated muscle including autosomal Emery-Dreifuss muscular dystrophy (EDMD), *LMNA*-associated congenital muscular dystrophy (L-CMD), and limb-girdle muscular dystrophy type 1B (LGMD1B). Here, we describe two novel *LMNA* gene mutations identified in two unrelated patients with congenital muscular dystrophy from Russia.

Two unrelated Russian families with myopathy of onset in the first year of life were investigated by us. Clinical features of patient 1 were: diffuse wasting with predominance in proximal upper and distal lower limbs, the spinal stiffness with head drop, thoracic hyperlordosisl, knee contractures and distal contractures in upper limbs. Clinical features of patient 2 with compromised perinatal history were: full-blown muscular hypotonia and psychomotor retardation. This patient died in seven month.

Mutation analysis was performed by direct DNA sequence on PCR fragments of each exons and exon-intron junctions of *LMNA* gene amplified from genomic DNA. In result previously not described deletion Glu31del in exon 1 was found in both families. Also previously not described change Ser334Asn in exon 6 of *LMNA* gene was found in patient and his mother from second family. However these both alterations were not found in 240 chromosomes of healthy persons.

Thereby the cause of congenital muscular dystrophy was de novo mutation Glu31del in exon 1 of *LMNA* gene. The mutations Ser334Asn had modificating effect for clinical severity of patient 2.

J12.24
Mutation analysis of MPZ gene in patients with Charcot-Marie-Tooth disease in Belarus

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The myelin protein zero (MPZ) gene is expressed in the compact layer of the Schwann cells. Mutations in MPZ gene lead to the CMT1B disease, and 77% of them are localized in exons 2 and 3, coding for the extracellular domain, indicating the importance of this domain for proper functioning of the MPZ protein.

At the first stage CMT1A and CMT1X were excluded in a group of selected patients with clinical symptoms of neuropathy. DNA resequencing analysis of exons 2 and 3 of MPZ gene revealed mutations in 2 of 50 (4%) probands with CMT. Missense mutation Thr124Met was observed in one patient having a clinically suggestive CMT2 phenotype characterized by late onset (>33 years) with papillary abnormalities (Argyll-Robertson pupils), deafness and chronic cough. In the second case we identified new mutation IVS1-2A>C associated with the severe motor and sensory neuropathy phenotype: early onset (during the first decade), muscular hypotonia, walking difficulties, distal weakness, sensory loss. This substitution was also identified in proband's father and her two children (boys of 2.5 years and 4 months). New mutation was not detected in healthy members of the family and normal subjects which supports the hypothesis that it is responsible for the CMT1B phenotype. These cases illustrate the importance of family studies in this disease, and establish genotype/phenotype correlation.

J12.25
Mutation in GJB2 in Iranian children with hearing loss referred for cochlear implantation

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Background: Mutation in GJB2 or Connexin 26 Gene is the most common mutation found in children with nonsyndromic hearing loss. Cochlear implantation (CI) is one approach to rehabilitation in such patients. Present study was designed for genetic assessment of children who were referred for CI.

Methods: Genetic analysis was performed on 42 Iranian children with nonsyndromic hearing loss who were referred for genetic consultation and CI. Genomic DNA was extracted from peripheral blood and the coding sequence of the GJB2 gene was PCR amplified and the products purified and directly sequenced.

Results: We found two homozygous and four heterozygous 35delG mutations in six of the patients (14.3%). The response to CI was better in the homozygous than in the heterozygoous patients.**Conclusion:** Mutation screening in *GJB*2 gene is a useful predictor of post-implantation speech perception.

J12.26
A novel GLA mutation H186P causatively linked to the Anderson-Fabry disease

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Background: The Anderson-Fabry Disease is an X-linked (Xq22) heritable disorder due to mutations in the GLA gene that causes a reduced or absent activity of the lysosomal enzyme alpha-galactosidase A. The enzyme defect leads to the intracellular and plasma accumulation of neutral glycosphingolipids, primarily globotriaoslyceramide.

This progressive accumulation of glycosphingolipids leads to the systemic disorder. This study demonstrates that the novel missense substution p.H186P is a pathogenic GLA gene mutation.

Methods: A 54-year-old male patient with unexplained concentric left ventricular hypertrophy and recurrent ventricular tachycardia, come to our attention. The screening of classical genes linked to hypertrophic cardiomyopathy (HCM) tested negative. We thus additionally tested the GLA gene.

Results: Bidirectional sequencing of the GLA gene showed that the patient was hemizygous for a single base sequence change in the codon 186 (p.H186P). The peripheral leukocytes showed a decreased GLA activity. Clinical and physical examination of the patient revealed the presence of cornea verticillata and angiokeratoma on the back.

Conclusion: Data in this study contribute to Fabry mutation database with a new mutation (p.H186P), confirming the reported heterogeneity in Fabry disease genotypes. The potential pathogenic effect of this novel mutation is likely due to conformational change in the enzyme structure leading to a less stable enzyme with a loss of activity.

J12.27
Receptor expression-enhancing protein 1 gene (SPG31) mutations are rare in Italian patients with hereditary spastic paraparesis

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Hereditary spastic paraparesis (HSP), is a large group of inherited, heterogeneous neurologic disorders caused by the degeneration of corticospinal axons.. Mutations in the *SPG4* and *SPG3A* genes, which encode for spastin and atlastin, respectively, are responsible for approximately 40% and 10% of cases of ADHSP, respectively. The *SPG31* gene also known as the receptor expression- enhancing protein 1 gene (*REEP1*), has been suggested to be the third most common cause of ADHSP, and accounts for 2.3%-8% of patients.

We report an investigation carried out on forty-five unrelated Italian HSP patients. All of the patients fulfilled the Harding criteria for the diagnosis of HSP. After giving informed consent, exhaustive *REEP1* mutation screening was performed on all of the patients by PCR and direct sequencing. No mutations in the coding region of *REEP1* gene were identified suggesting that the *REEP1* mutation frequency may be very low in Italian patients. Different studies carried out in European population indicate that mutations in *REEP1* account for up to 8% of autosomal dominant HSP cases. The low frequency of *REEP1* gene mutations detected in our population could be limited by factors such as PCR-based approaches that are sensitive to point mutations, as well as small deletions and insertions. A *REEP1*-specific multiplex ligation-dependent probe amplification (MLPA) assay is necessary to exclude multi-exonic *REEP1* deletion/duplication. Furthermore, the study of additional samples may increase the mutation detection rate.

J12.28
High incidence of W24X GJB2 mutation among Macedonian Gypsy patients with nonsyndromic hearing loss

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Hearing impairment affects 1 in 650 newborns, making it the most common congenital sensory impairment. Despite extraordinary genetic heterogeneity, mutations in one gene, *GJB2,* encoding the connexin 26 protein involved in inner ear homeostasis, are found in up to 50% of patients with autosomal recessive nonsyndromic hearing loss (NSHL). The spectrum of mutations in GJB2 varies considerably between different ethnic populations. 35delG predominates in Caucasians, 176delT in Ashkenazi Jews, 235delC in Japanese, while W24X in Indian and R142W in African population.

The aim of our study was to determine the prevalence and spectrum of GJB2 mutations, including the (GJB6-D13S1830) deletion, in Macedonian Gipsy patients with NSHL. Thirty unrelated patients (17 sporadic and 13 familial) of Gipsy origin, with profound deafness were analyzed. Molecular studies were performed using Single strand conformation polymorphism analysis, direct sequencing of GJB2 gene and specific PCR analysis for del(GJB6-D13S1830) mutation.

GJB2 mutations were found in 21 (70%) of the Gipsy patients, in homozygote (11), compound heterozygote (6) or heterozygote state (4). Among these the predominant mutation was W24X mutation with allelic frequency of 70.3%, followed by R127H and 35delG with allelic frequency of 16.2% and 10.8% respectively. In one patient a compound heterozygosity of W24X and Cd120delGAG was detected. None of the patients carry del(GJB6-D13S1830).

These findings indicate that as in the other Gypsy deaf populations the predominant GJB2 mutation is W24X as the major cause of autosomal recessive NSHL and should be tested in each routine diagnostic approach in Macedonian Gypsy deaf population.

J12.29
Association Of TNF-ALPHA Gene Polymorphisms And Inflammatory Bowel Disease In Iran

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Background and Aims: Assessment of tumor necrosis factor (TNF-α) gene polymorphisms in other countries, has shown an association with inflammatory bowel disease (IBD). Considering the genetic variety in different ethnic groups, the aim of present study was to investigate association of five important single nucleotide polymorphisms (SNPs) [-1031(T→C), -863(C→A), -857(C→T), -308(G→A) and -238(G→A)] in the promoter of the tumor necrosis factor (TNF-α) gene with inflammatory bowel disease in Iran.

Methods: Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods were used in this study to genotype DNA samples from 156 Ulcerative colitis (UC) patients, 50 Crohn's disease (CD) patients and 200 sex and age matched healthy controls.

Results: The frequency of the mutant (-1031C) allele of the TNF-α gene was significantly increased in Iranian Patients with Crohn's disease compared to healthy controls (26.0% vs 15.5%, P= 0.018, OR= 1.92; 95% CI: 1.14-3.23). No association was observed between other evaluated SNPs and Crohn's disease in Iranian patients. None of the mentioned SNPs were more frequent in Ulcerative Colitis patients compared to healthy controls.

Conclusion: TNF-α gene polymorphisms could partly play a role in disease pathogenesis in Iranian Crohn's patients.

J12.30
β-thalassemia mutations in the Iranian Kurds

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β-Thalassemia is the most common heredity disease in Iran and most part of the world. this disorder characterized by reduced (β+-thalassemia) or absence (β°- thalassemia) of the β- globin chain synthesis in Hemoglobin tetramer. Since the Iranian population is a mixture of different ethnic groups, it is necessary to determine the frequency and distribution of these mutations in our country. Therefore, in this study we determined the spectrum and the frequency of β-Thalassemia mutations in the population of Sunni Muslim Kurds in western Iran to set up a prenatal diagnostic laboratory.Genomic DNA was extracted by standard method. Mutations were studied in 120 chromosomes by Polymerase Chain Reaction- Amplification Refractory Mutation System (PCR-ARMS), and direct Sequencing methods. We found fifteen β- thalassemia mutations, and IVS-II-1 (G>A) mutation was the most common mutation with a frequency of 35%. Other mutations were FSC8/9 (+G) 15.7%, IVS-I-1(G>A) 8%, FSC8 (-AA) 6.75, FSC 5(-CT) 6.7%, IVS-I-110 (G>A) 6% , FSC 36/37 (-T) 4.2% , FSC 44 (-C) 3.4% , IVS-I-5 (G>C) 3.4% , IVS-I-6 (T>C) 3.4% , Codon 39 (C>T) 1.7% , Codon 15(T*G*G>T*A*G) 1.7% , IVS-I-128 (T>G) 1.7% , and +22 3'UTR (G>A) 0.8% , Codon 127(C*A*G>C*G*G) 0.8%+ 0.8%(one normal and one mutant allele).This report was the first comprehensive study in Kurdistan and western Azerbaijan provinces in Iran and could provide a reference for prenatal testing and genetic counseling in this population.

J12.31
Mutation detection of *GJB2* and *GJB6* and genetic linkage analysis of 3 common DFNB loci (DFNB4, DFNB3, DFNB59) in 9 large pedigrees with hearing loss in the Southern Khorasan in Iran

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Hearing loss is the most common sensory disorder. The autosomal recessive non-syndromic form (ARNSHL) accounts for 72% of monogenic HL. DFNB1 is the most common cause of ARNSHL in many populations (50%) including Iran (20%).Most of the research on HL in Iran has focused on this locus. The fact that many loci are

involved together with the heterogeneity of the status, necessitate studying further loci in various Iranian ethnic groups. Nine large deaf pedigrees originating from the Southern Khorasan province of Iran were selected. The families analyzed for *GJB2*, exon II, mutations. Affected heterozygous for GJB2 mutations were analyzed for two GJB6 deletions (D13S1830, D13S1854). Pedigrees negative for *GJB2* mutations were then subject to linkage analysis for DFNB4, DFNB3 & DFNB59 loci. Individuals were genotyped for STR markers using touch-down PCR-PAGE. Three out of the 9 families showed *GJB2* mutations. One family carried homozygous c.35delG mutation. The other pedigree carried two different mutations; One patient was heterozygous for c.231G>A while another carried a heterozygous c.380 G>A mutation. The second alleles were not detected. The 3rd pedigree showed to carry heterozygote mutation of p.V27I+E114G/wt . GJB6 deletions were not detected among the GJB2 heterozygous population. None of the remaining families showed linkage to the 3 other loci studied. Our study showed that *GJB2* mutations accounts for about 33% of affected families. These results could provide further insight into the etiology of HL and may lead to better genetic diagnostics & counseling. This study proceeds with more loci and more families.

J12.32
Cerebrotendinous Xanthomatosis (Sterol 27-Hydroxylase Deficiency) - A Case Report from Iran

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Cerebrotendinous xanthomatosis is a rare genetic metabolic disorder of cholesterol and bile acid metabolism that results in systemic and neurologic abnormalities. Mutations in the gene CYP27A1 were been known as cause of disease, more than 50 mutations have been implicated. Typically, the disease begins in infancy with chronic diarrhea. Cataracts become evident in childhood or adolescence, and xanthomata develop in the second and third decades of life. Significant neurologic impairment also occurs; this often includes seizures, dementia, and extra pyramidal dysfunction and typically begins in the third decade of life and progresses until death, often in the sixth decade of life if the condition goes untreated. The presentation and course widely varies, and treatment can dramatically alter the natural history, especially with early initiation.

Case Report We report here an Iranian family with two affected sibs who are suffering from Cerebrotendinous Xanthomatosis. Cardinal features were: Motor dysfunction, ataxia, spastic paresis. In the physical examination we found Xanthomas of the Achilles tendon and, ataxia, and cataract bilaterally. MRI of the brain showed diffuse cerebral atrophy and increased signal intensity in the cerebellar white matter on T2-weighted images**.** Cerebrotendinous Xanthomatosis, was confirmed by Molecular Analysis in this family. The result was a homozygous splice- mutation in intron 2 of the CYP27A1 gene.

J12.33
Analysis of the *CYBA* -930 A/G polymorphism (rs9932581) in sickle cell anemia patients and controls.

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Oxidative stress is a mechanism that might help to explain the phenotypic heterogeneity in the severity of sickle cell anemia (SCA) complications, due to vascular damage. The NADPH system is considered to be the most important source of super-oxide anion ($^{*}O_2^-$) in vascular cells such as smooth muscle and endothelial cells. The *CYBA* gene (16q24) encodes the essential subunit of the NADPH oxidase p22phox and has several allelic variants, some of them in the promoter region associated with higher transcriptional activity, as the -930A/G (rs9932581). Once there is no data regarding the frequency of this variant in the Brazilian population, it was determined in 135 SCA patients and 66 controls through direct sequencing. In the SCA group, the GG, AG and AA genotype frequency was 33.3%, 48.9% and 17.8%, respectively while in the control group it was 37.9%, 45.4% and 16.7%. The frequency of the G allele was 57.8% in patients and 64.4% in controls and of the A allele it was 42.2% in patients and 35.6% in controls, similar to the ones reported by the HapMap Project for CEU and ASW populations. There is no statistically significant difference in the genotype distribution between patients and controls although p value is equal to 0.053; the alleles are in Hardy-Weinberg equilibrium in both groups. These results suggest that there is no population subdivision in and between the studied groups and our population is suitable for further studies, as the association of the *CYBA* -930A/G polymorphism with severity in sickle cell anemia.

J12.34
Identification of genetic causes of Hearing loss in deaf GJB2 carriers in Iranian population

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Hereditary hearing loss is the most common neurosensory disorder in humans. Half of the cases have genetic etiology with extraordinary genetic heterogeneity. Mutations in one gene, GJB2, are the most common cause for autosomal recessive non-syndromic hearing loss (ARNSHL) in many different populations. GJB2 encodes a gap junction channel protein (connexin 26), and is located on DFNB1 locus on chromosome 13q12.11 which also involve another connexin gene, GJB6. Co-expression of GJB2 (connexin 26) and GJB6 (connexin 30) in the inner ear is thought to be prerequisite for the maturation of the cochlea. Mutation screening of GJB2 revealed that a high number of deaf patients carrying only one mutant allele.

In this study, using PCR-based direct sequencing; we searched for second mutant allele which leads to deaf phenotype. We assessed one hundred patients with ARNSHL through Iranian population bearing first mutation in their coding exon (exon-2) of GJB2, for mutations in non-coding exon of GJB2, promoter region (as the most important cis-regulatory elements) of GJB2 and GJB6, and 4 known deletions through DFNB1 locus. Passing the first step of study, to date we have identified the second mutant allele in splice site of exon-1 of GJB2 which is known as -3170G to A in 10 probands of 43.

Our findings confirm the results of last study on GJB2 mutations through Iran, which showed -3170G to A mutation as the most common after 35delG in Iranian population. Therefore, it is important to check GJB2 non-coding exon-1 in patients' heterozygote for GJB2 mutation.

P13 Metabolic disorders

P13.01
Mutation spectra of the AAAS gene in Iranian families with Allgrove syndrome

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Allgrove (OMIM#231550) or Triple-A syndrome is a rare, autosomal recessive disorder characterized by the triad of familial Adrenal insufficiency, Achalasia, and Alacrima. Approximately one-half of all patients with Triple-A syndrome have been shown to have mutations in the AAAS gene on chromosome 12q13, which result in loss or non-function of the encoded protein.

Five unrelated families clinically diagnosed as Allgrove syndrome were evaluated for sequence variations in the AAAS gene. Blood samples were collected after informed and written consent was received. Isolated DNA derived from subjects was amplified using intronic primers. The entire sequence of the AAAS gene including regulatory region, coding regions and exon-intron boundaries were analyzed for any alteration by PCR and direct sequencing.

In six probands of five families, four previously reported and two novel mutations were identified. Two heterozygote and homozygote mutations in exon 9 and the regulatory region respectively were

detected in one of the probands. In addition, SNPs analysis using SNPs plot's presented several previous reported variations in Allgrove families.
This is the first report of Triple-A syndrome from an Iranian population. Collectively, our study findings indicate that mutations scattered across the AAAS gene and upstream regulation elements. Various ethnic groups should develop a mutation database for their own rare genetic disorders. However, mutation databases should screen common mutated alleles initially.
Our families present the typical triad of symptoms and the mutation spectrum is similar to the other population studied. Further study is required for phenotype-genotype correlation in the Iranian population.

P13.02
Identification of Two Novel Mutations of *ASPA* Gene in Patients with Canavan Disease from Turkey

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Canavan disease (CD) is a childhood, progressive, autosomal recessive neurodegenerative leukodystrophy, characterized by macrocephaly, lack of head control, developmental delays by the age of three to five months, severe hypotonia, failure to achieve independent sitting, ambulation, or speech. It is prevalent in Ashkenazi Jews with two predominant mutations in affecting aspartoacylase (*ASPA*) gene. Its frequency in other populations is low with one predominant mutation, (914C->A).
We describe here three Turkish patients from two families. Two of them are siblings from first cousin parents. They had clinical features of poor head control, developmental delay, lack of visual tracking, progressive macrocephaly, limb spasticity and seizures which supported Canavan disease as a preliminary diagnosis. Neuroimagings were also compatible with CD. In addition, they had facial dysmorphic features including low set ears, up turned nostrils, long filtrum and retrognathi. Sequence analysis of *ASPA* gene revealed a novel homozygous donor splice site mutation (c.432+1G>A) in exon 2 of *ASPA* gene in both patients.
The third patient was 6 month-old boy from first cousin parents of Turkish ancestor. He was referred to our clinic because of progressive macrocephaly, nystagmus and poor sucking. Cerebral magnetic resonance imaging (MRI) and MR spectroscopy strongly suggested CD. Sequencing of *ASPA* gene revealed no abnormality while multiplex ligation dependent probe amplification (MLPA) analysis showed a novel large homozygous deletion in exon 1. In conclusion, our findings showed two mutations not previously reported in patients with CD suggesting new insights for the role of *ASPA* gene in this disease.

P13.03
Metabolic cutis laxa

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Cutis laxa is a rare skin disorder characterized by wrinkled, redundant, inelastic and sagging skin due to defective synthesis of elastic fibers and other proteins of the extracellular matrix. Wrinkled, inelastic skin occurs in many cases as an acquired condition. Syndromic forms of cutis laxa, however, are caused by diverse genetic defects, mostly coding for structural extracellular matrix proteins. Surprisingly a number of metabolic disorders are associated with inherited cutis laxa. Since the discovery of the COG7 defect, linking cutis laxa to glycosylation, several inborn errors of metabolism with cutis laxa have been described. In spite of the evolving number of cutis laxa-related diseases, a large part of the cases remains genetically unsolved.
Here we report on the clinical and metabolic features of 5 novel patients with ATP6V0A2-CDG, 4 children with PYCR1 defect and a young adult with RIN2 defect. In metabolic cutis laxa syndromes the clinical and laboratory features might partially overlap, however there are some distinct, discriminative features. We offer a practical approach to the differential diagnosis of metabolic cutis laxa syndromes.

P13.04
A new CDG1h case with a severe neurological and gastrointestinal presentation

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Congenital disorders of glycosylation type 1 (CDG1) are autosomal recessive conditions characterized by a distinctive serum transferrin glycoform pattern. The molecular basis is known for CDG1a - CDG1q. CDG1h (OMIM #608104) is associated with mutations in *ALG8* and nine cases have been published previously. Presentation of CDG1h varies from isolated protein-losing enteropathy to severe combined neurological and gastrointestinal disease.
We present a female, the second child of healthy consanguineous parents, born at 39 weeks with BW 2210g, OFC 33cm, L 48cm. Hypotonia was present neonatally and initial feeding difficulties persisted. At eight months findings included: very significant global delay, OFC at the 2,5%, growth retardation, horizontal nystagmus, poor eye contact, prominent chin, excess nuchal skin, wrinkling around ankles and knees. No specific abnormalities were noted on cerebral MRI. Serum isoelectric focusing was indicative of a CDG1. At age nine months, acute viral gastroenteritis was complicated by massive protein-losing enteropathy with rapidly ensuing lethal multi-organ failure.
SNP analysis using Affymetrix genome-wide human SNP array 6.0 revealed two known CDG1 associated genes, *MPI* and *ALG8*, within homozygosity regions in the proband only. Exome sequencing (Agilent 38Mb exome capture and 2x75bp Illumina sequencing) detected homozygosity for the *ALG8* variants p.S267P and p.F270L. Sanger sequencing confirmed parental heterozygosity. Both variants were interpreted as pathogenic based on conservation and three previously reported mutations predicted to affect the same intraluminal protein domain.
This case illustrates the severe CDG1h phenotype, and reinforces the clinical utility of homozygosity mapping followed by targeted exome sequencing in rare recessive disorders.

P13.05
Mutational Analysis of Uroporphyrinogen III Synthase Gene in Iranian Families with Congenital Erythropoietic Porphyria

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Porphyrias are rare metabolic hereditary diseases originating from defects in specific enzymes involved in heme biosynthesis pathway. Congenital Erythropoietic Porphyria (CEP) is the rarest autosomal recessive porphyria resulting from deficiency of uroporphyrinogen III cosynthase (UROS), the fourth enzyme in heme biosynthesis, leading to an excessive production and accumulation of type I porphyrins in bone marrow, skin and several other tissues. Clinical manifestations are presented in childhood with severe cutaneous photosensitivity, blistering, scarring and deformation in hands and loss of eyebrows and eye-lashes in face. Less than 200 cases CEP reported to date.
Two families with 3 CEP patients and members of their family in Iran were studied for the first time showing severe clinical symptoms including anemia, hepatosplenomegaly and severe photosensitivity along with scarring. A missense mutation in UROS gene was identified in this family. A T to C change at nucleotide 34313, leading to a substitution of Leucine by Proline at codon 237, which observed in

the homozygous state in 3 patients and heterozygous state in their parents. This family was conseled for any future prenatal diagnosis. Our data from Iranian population emphasize the importance of codon 237 alone in performance of UROS and advantages of molecular genetic techniques as a diagnostic tool for detection of clinically asymptomatic heterozygous mutation carriers as well as CEP within families.

P13.06
Long term follow-up and treatment in nine boys with X-linked creatine transporter defect

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The creatine transporter (CRTR) defect is a recently discovered cause of X-linked intellectual disability which is associated with cerebral creatine deficiency. Treatment with creatine monohydrate has not proved effective. Supplementation with creatine precursors L-arginine and glycine might increase endogenous cerebral creatine synthesis. The effect of this treatment is still controversial.

We followed nine boys aged between 8 months and 10 years with molecularly confirmed CRTR defect with repeated ^{1}H-MRS and neuropsychological assessments during 4-6 years of combination treatment with creatine monohydrate, L-arginine and glycine. Treatment did not lead to a significant increase in cerebral creatine content as observed with H^{1}-MRS. After an initial improvement of locomotor and personal-social IQ subscales, no statistical significant clinical improvement was recorded. Unrelated to the treatment, we noticed an age-related decline of IQ subscales in boys affected with the CRTR defect.

P13.07
Cystic fibrosis and tuberculosis

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Background: Cystic fibrosis patients are predisposed to acquire significant respiratory pathogens. Mycobacterium tuberculosis is a common pathogen in our area, having an important morbidity in children. Associated CF complication like underweight, diabetes mellitus and liver disease are well-known risk factors for tuberculosis. The hypothesis of a frequent co-morbidity of tuberculosis(TB) in CF children occurred. The aim of the paper was to evaluate the frequency of TB in children with CF in the presence of risk factors. Methods: Thirty-eight patients followed in our CF Center were considered for a prospective five years study. For the retrospective information we used data records of CF Centre. Diagnosis criteria for tuberculosis were: positive Mantoux test, BAAR positive and/or bacteriological confirmation, additional to clinical and radiological signs. Results: Regarding TB only one patient (2.6%) with CF had all the criteria diagnosis. Tuberculin skin test was positive in 4 patients (10.5%), 3 of them being vaccinated. Despite the mandatory vaccination for TB in our country, merely in 84% from patients the vaccination was documented. Interestingly, almost 16% of patients (6 pts) were considered and treated as TB cases, before being diagnosed with CF. One patient had active TB, bacteriological confirmed (del F508 homozygous, *Pseudomonas* positive and poor nutritional status). Conclusion: We could not find a correlation between tuberculosis and CF children, despite the presence of distinctive risk factors. Although TB is a quit common condition in our area, CF children seemed to be protected against it. Further studies need to be done to evaluate this hypothesis.

P13.08**
Identification and characterization of a novel inborn error of metabolism caused by Dihydrofolate reductase deficiency

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Our proband, born to consanguineous British Pakistani parents, presented at 3½ months of age with pallor and poor feeding. Investigations revealed megaloblastic anemia/pancytopenia with normal serum B12, folate and homocysteine levels. Within one week, he developed intractable seizures and cerebral folate deficiency was diagnosed. Brain MRI showed cerebellar and cerebral atrophy. His symptoms improved dramatically on folinic acid. His biochemical profile was consistent with reduced activity of dihydrofolate reductase (DHFR), a critical enzyme for synthesis of DNA precursors. DHFR has been studied extensively as target of anti-neoplastic, antimicrobial and anti-inflammatory drugs, but no cases of DHFR deficiency have been previously described.

Mapping identified multiple regions of homozygosity including a 3Mb region encompassing *DHFR*. Sequence analysis identified homozygous c.238C>T (p.Leu80Phe) mutation in the proband. Same homozygous mutation was identified in the proband's affected deceased sibling and in an unrelated child with similar clinical features. This mutation alters a highly conserved residue and was absent in 146 ethnically matched controls. It results in normal expression, but significantly reduced enzyme levels and activity. Protein modelling suggested that the Phe residue could disrupt cofactor binding and/or destabilize the protein.

Our proband continues to be severely neuro-developmentally delayed and was found to have cerebral tetrahydrobiopterin deficiency unresponsive to folinic acid. DHFR is important for maintenance of BH4:BH2 ratio in vascular endothelium and our findings suggest that DHFR may also facilitate BH4 salvage in the brain. The affected children had normal development until three months, indicating the possibility of better outcome with earlier diagnosis and treatment.

P13.09
Decreased INSR autophosphorylation due to novel p.Leu795Pro mutation in β-subunit in a patient with Donohue syndrome

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Donohue syndrome (former leprechaunism) is a rare, recessively inherited disorder of extreme insulin resistance due to mutation in the insulin receptor gene (INSR). The pathogenesis of insulin resistance is due to a cellular defect in insulin binding, receptor autophosphorylation and tyrosine kinase activity on insulin-stimulated biological activity. We have identified a newborn patient with Donohue syndrome caused by the novel homozygous missense mutation (p.Leu795Pro). The mutation is located in the extracellular portion of the β subunit of the insulin receptor. We aimed to functionally characterize the post-binding defect of the insulin receptor signaling in cultured fibroblasts from patient, patient's mother and control individual.

Functional characterization showed that novel INSR gene mutation in insulin receptor β subunit is causing a syndrome of extreme insulin resistance due to decreased insulin receptor autophosphorylation rather than insulin binding impairment.

P13.10
Familial Hypercholesterolaemia testing at the Bristol Genetics Laboratory

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Familial hypercholesterolaemia (FH) is an autosomal dominant disorder with UK incidence of approximately 1/500. FH is caused by mutations in the low-density lipoprotein receptor (*LDLR*), apolipoprotein B-100 (*APOB*) and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) genes.

A comprehensive FH testing service is offered at the Bristol Genetics Laboratory; including 1) ARMS PCR to detect 20 common mutations, 2) sequencing of *LDLR* (promoter + 18 exons) 3) MLPA of *LDLR* and

4) extended gene screening of *PCSK9* (12 exons) and *APOB* (codons 3375-3570) by sequencing.
Cases are referred through lipid clinics; genetic testing is offered to 'definite' and 'possible' FH cases (Simon Broome criteria in accordance with NICE). 156 index and 55 cascade cases have been reported to date. 46 different mutations have been identified, including a compound heterozygote for p.[Asp589fs]+[Lys311Thr]. A mutation was identified in 44% of index cases; of these 47% were detected using ARMS, 49% by *LDLR* sequencing and 4% using MLPA.
Genetic testing enables a definitive diagnosis, and defines the patient group requiring more aggressive treatment to lower cholesterol. Cascade testing for at risk relatives, provides an accurate risk outcome for cases where cholesterol measurement alone can be unreliable. Case studies demonstrating the clinical utility of molecular genetic testing for FH and an audit of the service to date will be presented.

P13.11

Enzyme replacement therapy with agalsidase alfa (Replagal) slows the progression of renal dysfunction in male patients with Fabry disease: results of an integrated analysis

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Introduction: Progressive kidney dysfunction is common in men with Fabry disease (FD). To estimate the effect of agalsidase alfa (agalα) enzyme replacement therapy on estimated glomerular filtration rate (eGFR), pooled data were analyzed post hoc from three similarly designed, prospective, randomized, placebo-controlled trials (RCTs TKT010/TKT005/TKT003).
Methods: 18-54-year-old males with FD received ≥1 infusion of agalα (0.2mg/kg; n=59) or placebo (n=58) every other week (EOW) for 6 months (analysis population). The Modification of Diet in Renal Disease (MDRD) equation was used to calculate eGFR in the analysis population at baseline and ≥3 follow-ups and in a subpopulation with chronic kidney disease (CKD) stages 2-3 (defined as eGFR 30−80mL/min/1.73m^2). Change in eGFR was calculated using a random slopes and intercepts model. This longitudinal analysis allows for serial measurements to exhibit within-patient correlations.
Results: Each RCT demonstrated numerical improvements in mean change in eGFR (agalα vs placebo). Pooled data showed statistically significant overall treatment differences (*P*=0.022), with kidney function deteriorated with placebo but not with agalα (although 95%CIs crossed 0; Table).
Conclusions: The results of this post hoc analysis of pooled RCT data support the hypothesis that agalα at a dosage of 0.2mg/kg EOW slows the progression of kidney dysfunction in male patients with FD.

eGFR rate of change during the 6-month treatment period.

	TKT010	TKT005	TKT003	All studies eGFR analysis population	All studies CKD 2/3
Placebo n/agalα n	39/37	8/7	12/14	59/58	21/20
eGFR: agalα, mean (95%CI), mL/min/1.73m^2/y	-8.53 (-19.08, 2.08)	1.72 (-29.54, 33.02)	11.60 (0.57, 22.57)	0.21 (-7.90, 8.27)	5.98 (-4.78, 16.69)
eGFR: placebo, mean (95% CI), mL/min/1.73m^2/y	-14.87 (-25.32, -4.47)	-17.11 (-46.44, 12.17)	-8.01 (-20.12, 4.06)	-13.26 (-21.48, -5.10)	-16.69 (-27.51, -5.88
eGFR: treatment difference (agalα - placebo)[a], mean (95%CI; *P*-value), mL/min/1.73m^2/y	6.40 (-8.48, 21.22; *P*=0.398)	18.88 (-24.02, 61.72; *P*=0.359)	19.60 (3.28, 35.93; *P*=0.019)	13.47 (1.98, 24.96; *P*=0.022)	22.67 (7.38, 37.91; *P*=0.005)

[a] Positive values favor agalα.

P13.12

First report of safety and tolerability of agalsidase alfa in Fabry disease patients formerly treated with agalsidase beta in a clinical trial setting

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Introduction: The FDA accepted a Treatment Investigational New Drug protocol (tIND; HGT-REP-059) allowing early access to agalsidase alfa (agalα) for patients with Fabry disease (FD) during the agalsidase beta (agalβ) supply shortage. This analysis describes safety and tolerability over 3 months in 70 FD patients switched from agalβ to agalα.
Methods: This ongoing, multicenter, open-label tIND collected 3-month data on adverse events (AEs), serious AEs (SAEs) and other clinical parameters from FD patients treated previously with agalβ. 117 patients enrolled, of whom, 59.8% (n=70) had prior treatment with agalβ, but not agalα. Excluded were patients who were pregnant, breastfeeding, hypersensitive to agalα, received any investigational drug (≤30days before entering the tIND) or were on concomitant agalβ therapy. Patients received intravenous agalα (0.2mg/kg every other week).
Results: 70 patients received study drug (ages 5-84 years, male:female 39:31). Among 68 patients with available data, mean prior agalβ therapy duration was 4.8 years (range 0.3-12.3). 76 treatment-emergent AEs (TEAEs) occurred in 30 patients. Most AEs were mild/moderate in severity (93.3%; 28/30 patients). No deaths occurred. The table summarizes possible/probable treatment-related AEs, infusion-related AEs, most common TEAEs (≥3% agalα-treated patients), treatment-emergent SAEs and AE-related permanent discontinuations.

Adverse event profile of 70 agalsidase alfa patients formerly treated with agalsidase beta.

Adverse event (AE)	Agalα-treated patients, % (n)
Treatment-emergent AEs	42.9% (30)
Possibly/probably treatment-related AEs	22.9% (16)
Infusion-related AEs	20.0% (14)
Most common treatment-emergent AEs (≥3% of all agalα-treated patients)	
-- Dizziness	4.3% (3)
-- Headache	4.3% (3)
-- Vomiting	4.3% (3)
Treatment-emergent SAEs	5.7% (4)
-- Cerebrovascular accident	1.4% (1)
-- Adverse reactions to antibiotic and skin abscesses	1.4% (1)
-- Transient ischemic attack	1.4% (1)
-- Diarrhea, vomiting, nausea, extreme fatigue, & dehydration	1.4% (1)
Permanent discontinuations due to AEs	0

Conclusions: Following 3-month evaluation of 70 FD patients previously treated with agalβ, agalα was generally well tolerated. These data support that agalα may provide a clinical option for FD patients who switch therapies.

P13.13

Multicenter, open-label treatment protocol (HGT-GCB-058) of velaglucerase alfa enzyme replacement therapy (ERT) in type 1 Gaucher disease: safety and tolerability

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Background: Type 1 Gaucher disease (GD1), an inherited deficiency of lysosomal glucocerebrosidase, is the most common lysosomal storage disorder. In 2009, the FDA approved a treatment protocol to offer velaglucerase alfa (then, an investigational drug) to GD1 patients affected by the imiglucerase supply disruption.

Methods: A US multicenter treatment protocol enrolled GD1 patients (>2 years old, treatment-naïve [naïve] or previously on imiglucerase [switch], with no prior anaphylactic reaction to ERT. Naïve patients received velaglucerase alfa 60U/kg every other week (EOW); switch patients received the same dose as their prior imiglucerase regimen (15-60U/kg EOW).

Results: From Sept 2009 through Jun 2010, 211 patients commenced velaglucerase alfa treatment (baseline: age 6-89 years [8 patients <18 years old]; 101 male:110 female; 72 splenectomized; 6 naïve; 205 switch). . One serious AE was considered possibly drug-related (migraine) [Table]. Median (range) velaglucerase alfa treatment duration for naïve and switch patients was 106 (27-232) and 182 (1-365) days, respectively. Mean hemoglobin and platelets improved in naïve patients (n=4; 1.7g/dL and 62.3 10^9/L) and remained stable for switch patients.

Adverse events (AEs)		
	Naïve	Switch
Description	N=6 n (%)	N = 205 n (%)
Experienced ≥1 AE	3 (50.0)	89 (43.4)
Experienced ≥1 possibly/probably drug-related AE	1 (16.7)	35 (17.1)
Experienced ≥1 infusion-related AE	1 (16.7)	27 (13.2)
Experienced ≥1 severe or life-threatening AE	0	11 (5.4)
Experienced ≥1 serious AE	0	7* (3.4)
Discontinued due to an AE	0	3 (1.5)
Deaths	0	0
*1 serious AE was considered possibly drug-related (migraine).		

Conclusion: A clinically heterogeneous group of GD1 patients were successfully transitioned to velaglucerase alfa, which was generally well tolerated, supporting this enzyme replacement therapy as a treatment option for GD1.

P13.14

Multicenter, open-label study of velaglucerase alfa enzyme replacement therapy (ERT) in type 1 Gaucher disease (GD1): Safety evaluation of the first three infusions from the HGT-GCB-058 treatment protocol

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Background: GD1, an inherited deficiency of lysosomal glucocerebrosidase, is the most common lysosomal storage disorder. In 2009, disruption of imiglucerase supply resulted in an FDA request for a treatment protocol to offer velaglucerase alfa (velaα) ERT (then, an investigational drug) for patients in need.

Methods: A US multicenter treatment protocol enrolled GD1 patients (>2 years old; no prior anaphylactic reaction to ERT). Treatment-naïve ("naïve") patients received velaα 60U/kg every other week (EOW); "switched" (previously on imiglucerase) patients received the same dose as their prior imiglucerase regimen (15-60U/kg EOW). The present analysis evaluates the safety profile of first 3 in-clinic infusions of velaα. Switched patients were monitored during these 3 infusions; if therapy was well-tolerated, their physicians could recommend home therapy infusions thereafter.

Results: From Sep 2009-Jun 2010, 211 patients were treated (baseline: age 6-89 years [8<18 years]; 101 male:110 female; 72 splenectomized; 6 naïve; 205 switched patients). The majority of possibly or probably drug-related AEs were infusion-related. The AE profile in switched patients was similar across the velaα dose range (15-60 U/kg). Fifty-four (26.3%) switched patients have received ≥1 home velaα infusion.

Adverse events (AEs) during the first 3 in-clinic infusions		
	Naïve	Switched
Description	N = 6 n (%)	N = 205 n (%)
Experienced ≥1 AE	2 (33.3)	51 (24.9)
Experienced ≥1 possibly/probably drug-related AE	1 (16.7)	25 (12.2)
Experienced ≥1 infusion-related AE	1 (16.7)	18 (8.8)
Experienced ≥1 severe or life-threatening AE	0	4* (2.0)
Experienced ≥1 serious AE	0	1† (0.5)
Discontinued due to an AE	0	1‡ (0.5)
Deaths	0	0
*3 severe AEs were considered drug-related (2 patients with fatigue; 1 patient with pain in the extremities) †Severe cerebrovascular accident (considered unrelated to treatment) ‡Discontinued due to a grade 2 infusion-related AE (nausea)		

Conclusion: In this clinically heterogeneous GD1 patient population, velaα was generally well-tolerated during the first 3 in-clinic infusions. This experience suggests early transition to home therapy with velaα is a treatment option based upon individual patients' responses and recommendations of their treating physicians.

P13.15

Clinical and genetic testing of further three patients with GSD I from Hungary

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Introduction: Glycogen storage disease type I (GSD I) comprises a group of rare, autosomal recessively inherited disorders with impaired glucose homeostasis due to deficiency of glucose-6-phosphatase activity. The most common form of GSD I, GSD Ia, caused by mutations in the G6PC gene leads to decreased or absent enzyme activity causing fasting hypoglycaemia, hepatomegaly, growth retardation, fasting lactic acidosis, hyperlipidaemia, and hyperuricaemia. The second most common type, GSD Ib, is caused by mutations in the G6PT1 gene and shows usually neutropenia and recurrent infections. In a previous study we reported phenotypic variability in 12 Hungarian GSD I patients. In this study we studied further three Hungarian children with GSD I.

Methods: Clinical diagnosis of all three children was GDS Ia. Liver biopsies were performed in all three cases. Genetic screening was carried out by PCR and sequencing of the G6PC and the G6PT1 genes.

Results: All children were clinically diagnosed as GSD Ia. In two children we found three mutations in the G6PC gene, of which one

is novel. In the third child we found a homozygous mutation in the G6PT1 gene.
Conclusions: Our results confirm the phenotypic variability of GSD I and point out the importance of genetic screening the G6PT1 gene in GSD Ia cases where no mutation in the G6PC was detected. Moreover, genetic testing might substitute liver biopsy. The novel mutation together with the other mutations found in the Hungarian GSD I patients can contribute to genotype-phenotype association studies.

P13.16
Egyptian glycogen storage disease type III - identification of six novel AGL mutations

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Background: Glycogen storage disease type III (GSD III) is caused by mutations in AGL which encodes for a single protein with two enzyme activities: oligo-1,4-1, 4-glucantransferase (transferase) and amylo-1,6-glucosidase. Activity of both enzymes is lost in most patients with GSD III, but in the very rare subtype IIId, transferase activity is deficient. Since the spectrum of AGL mutations is dependent on the ethnic group, we investigated the clinical and molecular characteristics in Egyptian patients with GSD III.
Methods: Clinical features were examined in five Egyptian patients. AGL was sequenced and AGL haplotypes were determined.
Results: Six novel AGL mutations were identified: a large deletion (c.3481-3588q1417del1525 bp), two insertions (c.1389insG and c.2368insA), two small deletions (c.2223-2224delGT and c.4041delT), and a missense mutation (p.L620P). p.L620P was found in a patient with IIId. Each mutation was located on a different AGL haplotype.
Conclusions: Our results suggest that there is allelic and phenotypic heterogeneity of GSD III in Egypt. This is the second description of a large deletion in AGL. p.L620P is the second mutation found in GSD IIId. Clin Chem Lab Med 2009;47:1233-8.

P13.17
The novel A175D mutation in SLC40A1 gene causes Ferroportin Disease in an Italian family

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Background. Type 4 haemocromatosis is a rare disorder of iron homeostasis, characterized by intracellular iron overload, hyperferritinemia and various levels of transferrin saturation, associated to mutations in ferroportin gene (SLC40A1), the sole known iron exporter in mammals. Loss of function mutations cause ferroportin disease (FD) (type 4a), whereas gain of function mutations cause a haemocromatosis clinically indistinguishable from that due to HFE gene (type 4b).
Aims and Methods. Aim of this study was to investigate the SLC40A1 gene in 86 patients with iron overload, in whom the mutation screening of HFE gene were negative and other primary causes of iron overload were excluded. All exons and 5'UTR region of SLC40A1 gene were analyzed by dHPLC and DNA sequencing. Phylogenetic analysis, prediction analysis (Polyphen) and homology modeling study (Swiss Prot) were performed for the novel detected variants.
Results and Conclusions. We detected the novel heterozygous missense variant c.524C>A (p.A175D) in three subjects of one Italian family. The mutation co-segregated with the affected family members and it was absent in 322 normal chromosomes. Interspecies comparison of ferroportin protein sequence showed that alanine 175 is strongly conserved. Software prediction and molecular modeling analysis indicate that its substitution with the negative charged aspartic acid 175 could modify the channel charge distribution, impairing the iron outing. This results indicate that the A175D is a novel loss of function mutation in SLC40A1 gene associated with ferroportin disease. A functional study for investigating in vitro the mutated iron exporter efficiency is underway.

P13.18
Hereditary Hemochromatosis in Russian children

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Hereditary Hemochromatosis type 1 (HH-1) is an inherited disorder of iron metabolism that results of mutations in the HFE gene. The most common mutations in HFE gene are C282Y, H63D and S65C. Since allele and genotype frequencies of these three variants of the HFE gene vary in different population, the determination of their prevalence in Russian children is important because genetic diagnostic in early stage can help to prevent the development of serious complications in these patients. There are two groups of children: 63 patients with biochemical signs of iron overload (BSIO, iron level was more 23,6 mkmol/l and transferrin saturation higher 40%) and 43 patients with somatic diseases without iron overload. All samples of DNA were genotyped by PCR followed by RFLP analysis. The H63D homozygote was identified in 14.2%, compound heterozygotes H63D/C282Y and H63D/S65C in 4.8% and 3.2% of patients with BSIO. The frequency of H63D heterozygote was in 3.5 times higher, than in control group (P=0,0004). We did not find patients with homozygote of mutations C282Y and S65C. However the frequencies of heterozygote mutations of C282Y and S65C in patients with BSIO were higher than in control group. Thus our data have shown that the main genotypes were homozygote H63D/H63D and compound heterozygotes H63D/C282Y and H63D/S65C in children with syndrome of iron overload. Taking in account that biochemical signs of iron overload syndrome could be observed in children with different somatic diseases therefore molecular-genetic study is necessary to confirm HH-1.

P13.19
Polymorphisms of interleukin-8 (rs2227543, rs4073) and -18 (rs1946518, rs187238) genes and diabetic retinopathy

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Diabetic retinopathy (DR) is caused by alterations of the retinal microvasculature leading to the breakdown of the blood-retina barrier and pathological angiogenesis. In DR and other angiogenesis-associated diseases, increased levels of cytokines, inflammatory cells, growth factors, and angiogenic factors are present. DR is one of the primary causes of visual loss worldwide.We evaluated possible roles of interleukin-8 gene polymorphisms (1633T/C-rs2227543, 251A/T-rs4073) and interleukin-18 gene polymorphisms (-607C/A-rs1946518, -137G/C-rs187238) in the development of DR in Caucasians with type 2 diabetes. 271 patients with DR and 113 without DR were enrolled in this cross-sectional study. We did not observe an association between either interleukin-8 gene polymorphisms (rs2227543, rs4073) or interleukin-18 gene polymorphisms(rs1946518, rs187238) and DR in Caucasians with type 2 diabetes. We did not find statistically significant differences in interleukin-8 serum levels between diabetics with the TT and AA genotype and those with other genotypes. The interleukin-18 serum levels between diabetics with the CC genotype of the rs1946518 polymorphism and those with other genotypes (AA, AC) were not significantly different. Moreover, we did not observe a statistically significant effect of the tested polymorphisms of either interleukin-8 or interleukin-18 genes on serum levels in diabetics. In conclusion, our study indicates that the examined polymorphisms of interleukin-8 (rs2227543, rs4073) and interleukin-18 (rs1946518, rs187238) genes are not genetic risk factors for diabetic retinopathy. Therefore, they may not be used as genetic markers for diabetic retinopathy in Caucasians with type 2 diabetes.

P13.20
Lesch-Nyhan syndrome in Russian patients

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Lesch-Nyhan syndrome (LNS) is an X-linked error of purine metabolism: hypoxanthine-guanine phosphoribosiltransferase (HPRT) deficiency produced by numerous *HPRT1* gene mutations.

The disease is associated with uric acid overproduction leading to early gout with renal and/or articular symptoms and neurological manifestations depending on the degree of enzymatic deficiency. In total HPRT absence classic LNS with severe movement disorders and self-injurious behavior develops. Partial HPRT deficiency, or Kelly-Seegmiller syndrome (KSS) produces phenotypes with less severe or no CNS involvement. While somatic symptoms are treated with allopurinol, there is no treatment for neurological symptoms. Mutations *de novo* make up 30 per cent of cases, most of patient's mothers are heterozygous carriers. Enzymatic and molecular diagnostics are in current use the latter being more reliable for carrier and prenatal testing. First Russian DNA-confirmed cases, three SLN and one KSS, are presented. In SLN patients age 3, 5 and 8 years three novel *HPRT1* mutations, Gly179Arg, c.606+1insT and IVS1+5G-A, were detected. Mutation Gly15Ser found in a 14-year-old boy with somatic KSS was described earlier with the same phenotype. All mothers and two patients' sisters proved to be carriers; in one family prenatal DNA testing was performed. Despite typical presentation, the diagnosis in all patients was significantly delayed, SLN was considered cerebral palsy and KSS was regarded as juvenile arthritis. That is, the disease is underestimated in practice. All boys with severe motor delay and hyperkinesias, even without self-injurious behavior, should be screened for hyperuricemia. Juvenile gout in males also needs testing for HPRT deficiency.

P13.21
Unravelling the leukodystrophies: clinical, biochemical and molecular aspects of sixty Brazilian patients with white matter abnormalities

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INTRODUCTION Leukodystrophies are a group of rare, heterogenous genetic diseases, which affect myelin, the major constituent of brain and spinal cord white matter.

AIMS Describe the clinical findings of sixty patients diagnosed with a genetic disorder of cerebral white matter and the approaches for the diagnosis.

METHODS/PATIENTS All the patients were evaluated in the neurogenetics clinics by geneticists and neurologists. Clinical anamnesis, physical exam, neuroimaging studies (CT scan and brain MRI with spectroscopy), ophtalmological and auditory evaluations, neurophysiological studies (EEG, ERG, BAER and EMG/NCV), hormone and biochemical tests, muscle biopsy with respiratory chain mitochondrial analysis, screening for inborn errors of metabolism (lysosomal enzymatic studies, peroxisomal and sterol panels, cholestanol dosage, organic acids, sulfatides and aminoacids chromatography, GAGs analysis) and, when indicated, nerve/skin biopsy for EM studies, karyotype and molecular tests were performed in the course the of investigation.

RESULTS Diagnoses in our patient population are summarized in Table 1

Disease group	Specific diagnoses (n)
Inborn errors of metabolism	X-linked adrenoleukodystrophy (4), MLD (3), Krabbe disease (2), infantile Refsum disease (1), α-methylacyl-CoA racemase deficiency (1), Niemann-Pick disease type C (4), Aicardi-Goutières syndrome (1), Canavan disease (1), neuronal ceroid lipofuscinosis (2), holocarboxylase synthetase deficiency (1), cerebrotendinous xanthomatosis (1).
Hypomyelination disorders	VWM disease (7; ovarioleukodystrophy, 1), Van der Knaap disease (1), 4H syndrome (1)
Mitochondrial disorders	MNGEopathy (1), Leigh syndrome (2).
Other disorders	Cockayne syndrome (1), leukoencephalopathy with brainstem and spinal cord involvment and lactate elevation (1), ARSAL syndrome (1), 18q deletion syndrome (1)

CONCLUSIONS About 50% of patients with white matter abnormalities remains without diagnosis even in a reference center after an exhausting investigation, therefore a specific protocol for studying and categorizing these patients is crucial not only for an academic or scientific purpose but it is mandatory for any clinician who wants to offer for such patients the correct therapeutics when it is available.

P13.22
Against All Odds: Treating Niemann-Pick type C in Brazil

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BACKGROUND: Niemann-Pick type C disease (NPC) is a rentless neurodenerative disorder characterized by a defect in cholesterol esterification causing lipid storage; Substrate Reduction Therapy (SRT) has been proposed as a treatment for NPC and other neurolipidoses based on the effects of iminosugars in the brain. OBJECTIVE: To report the follow-up of a cohort of Brazilian NPC patients treated with SRT for a period of 3 years

MATERIAL/METHODS: 28 patients with clinical, biochemical and/or molecular diagnosis of NPC were evaluated with a clinical protocol/ physical examination and complementary exams (abdominal ultrasound, brain MRI,EEG,NCV/EMG) during 3 years of treatment with the iminosugar N-butyldeoxynojirimycin (NB-DNJ)

RESULTS: All patients but two had started on NB-DNJ after the onset of the neurological symptons. Most of the patients have the classical childhood presentation of NPC disease, but there were 4 patients with perinatal presentation. Stabilization of mental deterioration was seen in 18 patients with neurological symptons - although in the remaining 8 patients NB-DNJ appeared to slow the neurological progression of the disease, further neurological deterioration could be seen. Electrophysiological studies did not demonstrate any peripheral neuropathy. NB-DNJ was only discontinued in two patients for different reasons (worsening of tremor and difficulty to manage the diarrhea episodes, respectively).

CONCLUSIONS: SRT seems to be a reasonable and safe approach to treat NPC patients. Nevertheless, there are patients that seem to have a poor response, regardless of the age of starting the treatment, so it is necessary to develop better biomarkers to follow treatment responses in NPC patients.

P13.23
Analysis of RNase P in MHBD deficiency

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Loss of function of the mitochondrial enzyme 17β-hydroxysteroid dehydrogenase type 10 (HSD10, MHBD) causes an organic aciduria with a neurodegenerative course and evidence of mitochondrial dysfunction. Several mutations in the *HSD17B10* gene have been identified. Interestingly, disease severity is not correlated to the dehydrogenase activity. Recently HSD10 was found to be part of the ribonuclease P (RNase P) protein complex in mitochondria. RNase P is an endonuclease consisting of three proteins (MRPP1, HSD10, MRPP3) and is responsible for the 5`-maturation of precursor transfer RNAs (pre-tRNAs) required for mitochondrial protein translation.

We now analyzed whether HSD10 mutations found in patients interfere with mtRNase P assembly or function. Quantitative RT-PCR analysis of different pre-tRNAs sequences in HSD10 knock-down cells caused accumulation of tRNA precursors. Western blot analyses of the three mtRNase P subunits in protein extracts from patient fibroblasts as well as HSD10 knock-down HEK and HeLa cells compared to control cells showed markedly reduced expression of both HSD10 and MRPP1. Expression of MRPP3 was not affected. On RNA level MRPP1 expression levels were comparable to controls, indicating that HSD10 mutations cause reduced MRPP1 protein stability. Surprisingly, pre-tRNA levels in patients` fibroblasts did not differ from control cells.

Taken together our results indicate that HSD10 mutations found in patients interfere with RNase P production, but this is not reflected in impaired mitochondrial tRNA processing in cultivated fibroblasts. It is possible that additional factors contribute to mitochondrial dysfunction in HSD10 (MHBD) deficiency.

P13.24
An APEX-based genotyping microarray for the screening of mutations in the LDLR gene

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Familial Hypercholesterolemia (FH) is an autosomal dominant inborn disease of lipid metabolism resulting in accelerated atherosclerosis and premature coronary heart disease. The early identification and treatment of FH patients is extremely important. FH is associated predominantly with mutations in the genes encoding the low-density lipoprotein receptor (LDLR) and its ligand apolipoprotein B (*APOB*). So far, more than 1000 sequence variants of the LDLR gene have been described. However, only one *APOB* mutation is prevalent in Europe.

Owing to time consuming and expensive classical DNA analysis of the whole LDLR gene (DNA sequencing), we have proposed a new diagnostic tool, an APEX (Arrayed Primer EXtension)-based genotyping DNA microarray called FH chip. The FH chip enables detection of 167 mutations in the LDLR gene and 1 mutation in the APOB gene simultaneously containing point mutations and small DNA rearrangements detected in Czech FH patients and other European and Asian FH populations.

To date, more than 300 FH suspected patients were analysed by using FH chip. In approximately 25 % of all cases a mutation was found, which corresponds to results obtained by DNA sequencing of the LDLR gene.

The FH chip is a rapid, reproducible, specific and cost-effective tool for genotyping and in combination with MLPA (Multiple Ligation-dependent Probe Amplification) represents a reliable molecular genetic protocol for the large-scale screening of FH mutations in the Czech population.

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P13.25
Exome sequencing, an efficient diagnostic tool for mitochondrial disorders

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Faulty energy supply due to defective oxidative phosphorylation is the biochemical signature of mitochondrial disorders a genetically heterogenous group of rare, severe and highly invalidating human conditions. Due to it's clinical and genetic heterogeneity the diagnosis of mitochondriopathies is challenging and usually relies on biochemical analysis of biopsy material. Therefore, the majority of patients lack a molecular diagnosis and there is an urgent need for new strategies to close the gap between biochemical definition and molecular genetic correlate.

Next generation sequencing strategies are currently revolutionizing mutation detection both for the routine diagnosis of known mutations and the identification of new genes for rare disorders.

We demonstrate the efficacy of exome sequencing in combination with a cellular rescue-assay to elucidate the genetic basis in biochemically defined mitochondrial disorders. We report the identification of mutations in known disease genes and the discovery of causative mutations in ACAD9, a member of the acyl-CoA dehydrogenase (ACAD) protein family. The pathogenic role of novel variants was established by the correction of the biochemical defect on expression of the wild-type protein in patient's fibroblasts. ACAD9 screening of 120 patients identified two additional index cases.

Riboflavin, the vitamin precursor of the FAD moiety, which is the catalytic cofactor of ACADs, is known to foster their assembly and stability. Riboflavin treatment of mutant cell lines for 3 days resulted in a significant increase of complex I activity. Notably, recent publications and our own clinical observations support beneficial effects of a high dosage riboflavin regimen in ACAD9 patients.

P13.26
Screening for mitochondrial deafness mutations in Hungarian patients: experience of 7 years

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Sensorineural hearing loss is the most common phenotype of mitochondrial diseases. Although in most cases interfamilial and intrafamilial phenotypic variation is observed, the clinical presentation can be specific for a certain mutation. The mutations reported so far affect mitochondrial tRNA and 12S rRNA genes. Our department performs mitochondrial DNA analysis since 1999 and mutation screening of nuclear and mitochondrial deafness-genes since 2003. Among 60 Hungarian patients affected by hearing loss we identified one common syndromic deafness mutation and two rare Ser^{UCN}-tRNA mutations in two families with non-syndromic hearing impairment. In a 12-year-old boy with seizures, bilateral hearing loss and ragged red fibers the A3243G heteroplasmic mutation was detected. In a 10 year-old boy and his family with bilateral hearing loss but no palmoplantar keratoderma we identified the A7445G Ser^{UCN}-tRNA mutation in homo- and heteroplasmic forms independently of the hearing status, delineating our case from other pedigrees. Haplotype analysis revealed a rare variant of the U4b haplogroup. In a 5-year old boy and three generations with bilateral hearing loss analysis revealed the T7510C Ser^{UCN}-tRNA mutation in homoplasmic form, reported only in 3 Caucasian families previously.

According to the incidence of mitochondrial diseases the role of mtDNA mutations in hearing loss is expected to be higher as verified. Among 60 patients we found two rare mutations both affecting the Ser^{UCN}-tRNA. Since in some families maternal inheritance is not clearly revealed, screening for mitochondrial mutations should always be considered in any family in which paternal transmission of the hearing impairment can be excluded.

P13.27**
Mutations in TTC19 cause mitochondrial complex III deficiency and neurological impairment in humans and flies.

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Deficiencies of Mitochondrial Respiratory Chain complex III (cIII) are rare and can be caused by mutations in mtDNA-cytochrome b or in nuclear BCS1L genes; nevertheless most of cIII-defective cases remain genetically undefined.

We identified a homozygous non-sense mutation in the tetratricopeptide 19 (*TTC19*)-encoding gene in patients from two families, affected by progressive encephalopathy associated with profound cIII deficiency and accumulation of cIII-specific assembly intermediates. A second homozygous nonsense mutation was later found in a fourth unrelated patient. We experimentally demonstrated that the wild-type TTC19 protein is targeted to, and embedded in, the inner mitochondrial membrane. We then showed that TTC19 is part of two high-molecular weight complexes, one of which co-migrates with cIII. Physical interaction between TTC19 and cIII was then demonstrated by co-immunoprecipitation.

By in silico data mining, TTC19-like protein sequences were found throughout the Metazoan radiation. We investigated a *Drosophila melanogaster* knockout model for TTC19 that showed low fertility, strong bang sensitivity, severely reduced spontaneous motor activity, and impaired optomotor test, associated with cIII deficiency. Importantly, the cIII deficiency found in the KO adult flies was not present in mutant larvae, that developed normally into adult individuals, suggesting the existence of an alternative cIII assembly process in the developing stages of the fly.

In conclusion we provide evidence that TTC19 is a putative cIII

assembly factor in animals, and its absence determines severe neurological abnormalities in both human and flies.

P13.28
Novel mutation in *MPV17* gene in two sisters with hepatocerebral mitochondrial depletion syndrome.

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Mitochondrial DNA depletion syndromes (MDSs) are autosomal recessive disorders characterized by tissue-specific reduction of mtDNA copy number leading to dysfunction of affected organs. They usually develop in infants and small children, and are clinically classified as myopathic, encephalomyopathic and hepatocerebral forms. The cases, where liver was the most or the only affected organ, usually die of severe liver failure before 1 year of life. At least 10 nuclear genes are known to be involved in the pathogenesis of particular MDS form.
Here we describe two sisters presented with progressive liver failure (cholestasis, jaundice, elevated liver enzymes, hepatomegaly, iron accumulation), encephalopathy (hypotonia, nystagmus, periodical stridor), poor feeding and lactic acidosis started from a very beginning after birth, died in 12th and 7th month of life.
Histological picture of liver biopsy of an older sister and autopsy in the younger one was characteristic for hepatocerebral MDS. Severe mtDNA depletion was detected in liver (89%) and muscle tissue (83%) of the younger girl, but was not so evident in muscle sample of the older one (60%, liver-NA).
Molecular analysis of selected genes associated with hepatocerebral MDS (*DGUOK, MPV17, POLG1*) revealed a novel mutation c.191C>G (p.Pro64Arg) on two *MPV17* alleles in both sisters confirming clinical diagnosis of hepatocerebral MDS. We consider this molecular variant to be pathogenic as it is located in conserved transmembrane domain of the protein.
It is the first report of mitochondrial depletion associated with *MPV17* dysfunction in Polish patients.
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P13.29
The clinical variability of mitochondrial encephalomyopathy due to mutation in SCO2 gene

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The cytochrome C oxidase (COX) - complex IV of the respiratory chain consists of 13 protein subunits encoded by mitochondrial (3) and nuclear (10) DNA. The low activity of complex IV is caused not only by mutations in genes coding particular subunits but also by mutations in other genes of both mitochondrial and nuclear DNA. One of them is SCO2 gene. Mutations in this gene resulting in a defect in COX assembly, lead to a lethal infantile cardioencephalomyopathy. In the clinical picture dominate: muscular hypotonia leading with time to respiratory failure, limitation in extraocular movements, hypertrophic cardiomyopathy, lactic acidosis, decrease of COX activity in muscle biopsy.
The authors analyze clinical features in three infants with the mutation in SCO2 gene. As the first symptom all the children had muscular hypotonia with hypo/areflexia. Progress of clinical symptoms leading to respiratory insufficiency was observed. At one of the children in the following stage of the disease laryngeal stridor and symptoms of laryngomalacia were dominating. Cardiomyopathy appeared in the final stage of the disease suggesting mitochondrial myopathy. As for the other two children in the initial stage, both clinical symptoms and neurogenic pattern in EMG suggested spinal muscular atrophy. The following symptoms: hyperlactatemia, ptosis, and changes in MRI in one of them, suggested mitochondrial cytopathy without cardiomyopathy. For all the children mutation in SMN1 gene was excluded and next neurogenic atrophy in muscle biopsy was stated. Molecular study of SCO2 gene revealed the same homozygous p.1541G->A mutation and allowed the final diagnosis.

P13.30
A novel mitochondrial ATP6 frameshift mutation causing isolated complex V deficiency, ataxia and encephalomyopathy

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Background: The process of ATP production by mitochondrial ATPase (F1F0-ATP synthase, complex V) is a rotary process driven by an electrochemical proton gradient across the inner mitochondrial membrane. F0 that couples the proton gradient to ATP synthesis by F1, contains among others the subunits ATP6 and ATP8 that are encoded by mitochondrial DNA. Dysfunction of mitochondrial ATPase due to missense mutations in ATP6 is a frequent cause of severe mitochondrial encephalomyopathies.
Purpose: To identify the biochemical and molecular defect in a 4 year old girl with ataxia, microcephaly, developmental delay, mental retardation and elevated lactate suspected for a mitochondriopathy.
Methods: OXPHOS measurements were performed in patient's skeletal muscle and skin fibroblasts. BN-PAGE with subsequent immunoblotting was applied to study complex V assembly. Relevant parts of the mitochondrial genome were sequenced. The function of the respiratory chain was analysed by high resolution respirometry.
Results: We found an isolated reduced complex V activity in the patient's muscle tissue and fibroblasts. Immunoblotting after 1D BN-PAGE revealed in addition to the complex V holocomplex increasing amounts of mitochondrial ATPase subcomplexes. Oxygen consumption studies in patients fibroblasts demonstrated reduced ADP-stimulated respiration with pyruvate/malate or succinate as substrates. A heteroplasmic frameshift mutation was found in the ATP6 gene in patient's muscle tissue and blood. The mutation was not detectable in the mother's DNA extracted from blood and buccal cells.
Conclusion: We describe a novel pathogenic frameshift mutation in ATP6 associated with ataxia and encephalomyopathy resulting in incomplete assembly and reduced activity of the complex V holoenzyme.

P13.31
A family case of Leigh syndrome caused by a novel mutation in mtDNA

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A family case of Leigh syndrome with atypical manifestation caused by a novel mtDNA mutation is presented. The family consists of a mother and her two sons: 7-year-old and 4-year-old boys. The delivery of both brothers passed with no serious complications. Early motor development was normal. At age of 2.5 years, against the absolute health, in both children a restrain of right hand was noticed. Since that time the disease has an undulating course with progressive hyperkinesis, dystonia, dextral hemiparesis and dysarthria. Speech and mental development is delayed and worsenes. Expressive language is poor but receptive language is satisfactory. There were no seizures, vision or hearing problems. MRI of elder brother showed symmetric lesion of basal ganglia, but MRI of junior brother revealed the same damage only on the left side. MRI of mother showed similar damage on the right side, however she has only moderate sinistral hemiparesis since the age of 5 years, when a clumsy left hand was noticed.
Leigh disease was supposed. After *SURF1* gene mutations were excluded, a search of mtDNA mutations in blood cells was performed. A novel substitution m.3945 C>A in ND1 gene was detected in children in homopasmic state and in heteropasmic state in the mother.
A nucleotide replacement leads to substitution of aminoacid Ile to Met. In 212 control DNA samples the same substitution was not found. We proposed this substitution being pathogenic.
Thus, this case demonstrates the clinical variability of Leigh syndrome and proves the necessity of mtDNA sequencing.

P13.32
Clinical diagnosis and management of patients with mucopolysaccharidosis type II in Belarus

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Mucopolysaccharidosis type II (MPS II, Hunter syndrome) is a multisystem, multiorgan X-linked recessive disorder with a variable age of onset and variable rate of progression caused by deficiency of the lysosomal enzyme iduronate-2-sulphatase.

Aims: To summarize the clinical features of patients with MPS II in Belarus and to characterise the dynamic of the disease progression for the improvement of the clinical diagnosis.

Methods: We present the clinical observation of Hunter syndrome cases which were confirmed by laboratory biochemical methods. The clinical study includes the analysis of 139 phenotype features: facial abnormalities, neurologic, ophthalmologic, auditory, cardiovascular, respiratory, gastrointestinal, musculoskeletal symptoms etc.

Results: 18 patients (males) with MPS II from 14 unrelated families were studied. Median age at diagnosis was 5 years 1 month (range: 1-23,5 years), median age at onset of symptoms was 3 months (range: at birth â€“ 2,5 year). Interval from symptom onset to disease diagnosis was 4 years 1 month (range: 1,9â€“23,3 years). Four patients were died, two of them at the first decade and two another at second decade of life, median age of death was 12 years 1 month (range: 7â€“19,4 years). Basing on the analysis of facial abnormalities, neurologic, ophthalmologic, auditory, cardiovascular, respiratory, gastrointestinal, musculoskeletal symptoms we defined the most significant phenotypic markers and pattern of early clinical diagnostics and developed management of this disorder in Belarus.

P13.33
Prenatal cerebral cysts: presentation of the first prenatally diagnosed Molybdenum cofactor deficiency (MOCD)

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Molybdenum Cofactor Deficiency (MOCD) is a rare autosomal recessive metabolic disorder, caused by a disrupted function of sulfite oxidase (SO) that requires MOC for proper functioning.

Case 1

After a full term pregnancy of healthy Caucasian non-consanguineous parents, a girl was born by emergency caesarean section. Amniotic fluid contained thick meconium . Six hours postpartum she developed seizures. Care was withdrawn five days after birth because of severe brain damage. The microscopic abnormalities in the brain were compatible with severe peripartum asphyxia.

Case2

Ultrasound examination in the second pregnancy at 34 weeks of gestation showed bilateral cerebral cysts, ventriculomegaly and cerebellar hypoplasia. On suspicion of a SO deficiency or MOCD an amniocentesis was performed, showing high S-Sulfocysteine in the amniotic fluid. Delivery was induced and a macrosomic girl was born with full cheeks and deep-set eyes. MOCD was confirmed in urine, plasma and amniocytes postnatally. Beyond the neonatal period she developed seizures. Brain MRI showed progressive cerebral atrophy. She died at the age of 3 ½ months.

Sequence analysis of MOCS1 showed a homozygous splice site mutation (c.418+1 G>A) in both cases and heterozygosity in their parents.

Conclusion

These sibs display the variable expression of MOCD, ranging from severe asphyxia with neonatal convulsions to extensive cerebral deformations. Metabolic work-up, including measurement of S-sulfocysteine, should be performed in a fetus with prenatal brain cysts and newborns with unexplained asphyxia or brain damage to make a timely diagnosis and offer parents the opportunity of prenatal diagnosis or PGD in a future pregnancy.

P13.34
Clinical and molecular data of 24 patients with mucopolysaccharidosis type VI from Poland, Baltic States and Belarus

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Background. Mucopolysaccharidosis type VI (MPS VI; Maroteaux-Lamy syndrome) is a rare autosomal recessive disorder caused by a deficiency of N-acetylgalactosamine-4-sulfatase (ARSB).

Aim. We aimed to analyze the natural history and the spectrum of mutations responsible for the disorder in Central and Eastern Europe.

Material and Methods. We studied 20 unrelated MPS VI families (4 Polish, 6 Lithuanian, 2 Estonian and 8 Belarussian), in whom clinical diagnosis was biochemically confirmed by demonstrating abnormal excretion of dermatan sulphate in urine and deficient activity of ARSB in plasma or fibroblasts.

Results. Clinical heterogeneity was observed among our patients and three major clinical phenotypes of the disease can be distinguished: severe (n = 6, 25%), attenuated (n = 9, 37.5%) and intermediate (n = 9, 37.5%). A remarkably large number of patients in our cohort showed an attenuated phenotype of the Maroteaux-Lamy syndrome, with regression later in life and prolonged survival. We identified 97.5% of the ARSB mutant alleles, 6 of them novel (c.31091insCCTGAAG+delATACT, Q88X, T92K, W57C, G167R and 161_166insT). We also report 5 previously described mutations (R152W, Y210C, Y266S, G302R, Q97X) as well as 2 non-pathogenic polymorphisms (A33V, S384N).

Conclusions. 1. Three major clinical phenotypes of the disease could be distinguished among our patients: severe, attenuated and intermediate. 2. When compared with data published in the literature, a larger number of patients in our study presented with an attenuated phenotype. 3. The relatively attenuated phenotype may be associated with the R152W mutation, which suggests a specific genotype-phenotype correlation.

P13.35
Molecular analysis of most common mutations in Phenylalanine hydroxylase gene in Iranian population

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Phenylketonuria (PKU) is the most prevalent disorder of amino acid metabolism. It is one of the most important preventable causes of mental retardation. Incidence of PKU in Iran has been estimated at 1 in 3627 births. The aim of this study is to assess the prevalence of PKU mutations in Iranian population. For this purpose, 150 unrelated patients with classic PKU (300 alleles) were screened for 10 mutations (IVS10-11g>a, R252W, R261X, R261Q, IVS11nt1, R408W, R408Q, L333F, 364delG and S67P) using polymerase chain reaction-restriction fragment length polymorphism. The predominant mutations in this population sample are IVS10-11g>a, R261Q, IVS11nt1 and R252W with the frequency 21.7%, 9%, 6.7% and 4.7% respectively. In addition, 6 other mutations have been identified at relatively low frequencies (R261X (4%), 364delG (3.7%), L333F (2%), R408W, R408Q and S67P (0.33%)).

These informations provide a good basis for direct DNA diagnosis of PKU in this population.

P13.36
Osteoclasts derived from patients with NF1 display in vitro tolerance to zoledronate

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Neurofibromatosis type 1 (NF1) is an autosomal dominant neuro-cutaneous-skeletal syndrome with an incidence of about 1/3000. Up to half of patients with NF1 have osteoporosis. Bisphosphonate drugs are used in osteoporosis, and the drug results in reduced small GTPase activity (including Ras) in osteoclasts, ultimately leading to slower bone remodeling. As NF1 is associated with hyperactive Ras, our aim was to evaluate in vitro effects of zoledronate in NF1 osteoclasts. Bone mineral density (BMD) of 16 patients with NF1 was measured and their osteoclast progenitors were isolated from peripheral blood. Control osteoclasts were obtained from 16 age- & sex-matched control persons.

Osteoclast progenitors were differentiated into osteoclasts in vitro, and were treated with 10E-6, 10E-7 and 10E-8 zoledronate. NF1-osteoclasts demonstrated a dose-dependent tolerance to zoledronate compared to control osteoclasts. Three patients were taking bisphosphonates at time of the study, but no differences in their cell culture results were found. The in vitro results may reflect to long-term effects of bisphosphonates in osteoporotic patients with NF1.

P13.37
Second tier testing by tandem mass spectrometry reduces the false positive rate in newborn screening (NBS)

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The primary goal of newborn screening is the early diagnosis of asymptomatic newborns affected with treatable conditions. The desire for the highest possible sensitivity has the innate risk of compromising specificity thus not only adding to the cost of management of the patients but also potentially increasing the risk of dysfunctional parent-child relationships. Approaches to reduce false positive results include: 1) utilization of as many as possible analytes and informative ratios; 2) Definition of clinically validated cutoff target ranges; 3) Implementation of second-tier tests (performed on the same specimen without further patient contact).

In recent years our laboratory has introduced in routine clinical practice the following second-tier tests: 1) profiling of methylcitric acid, methylmalonic acid and homocysteine for disorders of propionate and methionine metabolism; 2) succinylacetone for Tyrosinemia type I (now incorporated in the primary screening); 3) alloisoleucine for maple syrup urine disease; 4) steroid profiling for congenital adrenal hyperplasia (CAH); 5) Ethylmalonic acid, glutaric acid, 3-hydroxyglutaric acid and 2-hydroxyglutaric acid for Glutaric acidemia type I and Type II.

Utilization of these methods has impacted our practice in three ways: First, the false positive rate of screening by MS/MS has declined to 0.05 % with a positive predictive value of 64 % (2010 metrics); Second, problematic cut-off values have been optimized to reduce the risk of false negative results; Third, we are able to screen for new groups of disorders, not previously included in the screening panel such as remethylation disorders and different types of CAH.

P13.38
Niemann-Pick disease, type C: phenotypes and *NPC1* mutations in Russian patients

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Niemann-Pick disease type C (NPC) is an autosomal recessive neurovisceral metabolic disease, one of sphingolipidoses. NPC is heterogeneous genetically and clinically. About 95% of cases are caused by numerous mutations of *NPC1* gene. Juvenile NPC manifesting in 4-14 years, usually before 10 years, makes up about 65% of cases; infantile, early childhood and adult forms are infrequent. Genotype-phenotype correlations exist. Along with DNA testing detection of non-etherified cholesterol in skin fibroblast lysosomes is in current use, but it is more complicated and less reliable for prenatal testing. Recently pathogenetic treatment with miglustat was proposed which makes early diagnostics vital. We diagnosed NPC in five unrelated patients, in one of them the diagnosis was verified biochemically, in four others by DNA testing (in two cases only one of allelic mutations was detected). Four children age 5-9 years, three of Russian and one of Azerbaijan ethnicity, had severe juvenile NPC with dementia and multiple neurologic signs in all and evident visceromegaly in two. Adult NPC in a 18-year-old Byelorussian girl presented mostly with dementia and psychiatric signs including acute psychosis while neurologic signs and splenomegaly were mild and transient which, along with normal MRI, hampered diagnostics. The genotypes were: p.Pro1007Ala/?, p.Ser954Leu/c.3614delC, p.Ser954Leu/? (in Azerbaijan patient) and p.Ser954Leu/c.326dupT (in adult NPC). Mutation p.Pro1007Ala is relatively common in European populations (along with p.Ile1061Thr); mutation p.Ser954Leu was described previously in German and Canadian patients in heterozygous or compound heterozygous state like in ours; mutations c.3614delC and c.326dupT are novel.

P13.39
Correlation between lipid profile / cardiovascular risk and type of mutation in Portuguese patients with familial hypercholesterolemia

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Familial Hypercholesterolemia (FH) is characterized by high levels of LDLc in plasma, accelerated atherosclerosis and increased risk of premature coronary heart disease. FH results from mutations in three genes involved in lipid metabolism: LDLR, APOB, PCSK9. It is known that FH patients' phenotype is heterogeneous varying with different conditions.

The aim of this study was to analyse the biochemical profile of patients with genetically diagnosed FH in accordance with the mutations in different genes and the different types of mutations identified in the LDLR gene to identify whether there is a correlation between these variables in Portuguese patients.

Biochemical parameters, total cholesterol (TC), LDLc, HDLc, triglycerides, ApoB and ApoA of 325 patients with FH were analyzed by SPSS using ANOVA and Tukey.

Patients with mutations in the PCSK9 have LDLc and TC levels significantly higher than patients with mutations in the remaining two genes. There is no significant difference in these parameters between patients with mutations in LDLR and APOB genes, although all values are higher in patients with mutations in the LDLR. Patients with nonsense mutations in the LDLR gene present values of TC, LDL and ApoB statistically higher than in patients with missense mutations. The comparison between the other categories showed no significant changes in biochemical parameters. Patients with mutations in the PCSK9 have a more severe phenotype than patients with mutations in LDLR and APOB genes.

The type of mutation (in different genes or different mutations in LDLR) is important in determining cardiovascular risk in FH patients.

P13.40
Susceptibility genes of metabolic syndrome and its symptoms in children and adolescents with obesity

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The prevalence of metabolic syndrome (MS) in developed countries, particularly in children, continuously grows. The aim of our study was to assess frequency of occurrence of polymorphisms of candidate genes TCF7L2, APOE, APOC3, LPL, REN, ADRB1, ADRB2, AGTR2, ACE, AGT, BKR2, NOS3, MTHFR in children with metabolic syndrome and control group.304 children were enrolled in this study: 160-main group (patients with obesity) and 144-control group in the age of 5-17 years (mean age was 12±0,18). MS was diagnosed in accordance with criteria, proposed by IDF in 2007. All polymorphisms were determined by PCR-RFLP and PCR-biochip methods. MS was diagnosed in 28

(17,5%) children in the age of 11-17 years. We observed an increase of TCF7L2 C/T and T/T genotype frequency (58,2% and 33,0%; p<0,001) in children with obesity, in comparison to healthy children. Significant differences were found in ADRB2 C/G and G/G genotype distribution (52,5% and 18,6%; p=0,003) between obese patients and control group. Children with obesity possessed significantly higher AGTR2 A/A genotype frequency (37,3%; p=0,019) in comparison to healthy children, besides higher A/A genotype frequency was observed among boys (69,0%; p<0,001). NOS3 T/T genotype was more frequent in girls than in boys (55,1%; p=0,031). ACE D/D genotype was more frequent in boys (57,0%; p<0,05). REN A/A and A/G genotypes were more frequent in boys (11,1 and 26,7%; p=0,021). During the selection of risk groups MS for children with obesity it is expedient to analyses for a patient polymorphism of genes of TCF7L2, ADRB2, AGTR2.

P13.41
Variation in DNA methylation influences metabolic syndrome-related phenotypes

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Metabolic syndrome is a complex disorder that is reaching pandemic proportions and is a strong predictor of diabetes and cardiovascular disease. Currently little is known about the importance of epigenetic variation in disease risk. We performed preliminary analysis quantifying relative DNA methylation in 190 Mexican Americans, within the San Antonio Family Heart Study, at 1,505 gene-centric CpG sites using an Illumina GoldenGate assay to identify epigenetic correlates with metabolic syndrome-related phenotypes. Linear models were used to identify a number of consistent correlations across multiple dimensions of metabolic syndrome for many CpG sites in highly relevant genes. For example, CpG methylation within *POMC* was correlated with fasting (p=0.031) and 2 hour (p=0.020) insulin, high density lipoprotein cholesterol (HDL-C; p=0.002) and triglycerides (p=0.009) as well as with NCEP:ATPIII defined metabolic syndrome (p=0.011). Deficiencies in *POMC* are strongly correlated with obesity and it is possible that methylation reflects another mechanism by which decreased expression influences body weight. Methylation of CpG sites in other candidate genes such as *RYK*, *RUNX1T1* and *TGFBR3* were also correlated with various metabolic syndrome phenotypes including fasting and 2 hour glucose, fasting and 2 hour insulin, body mass index, waist circumference, HDL-C, triglycerides and metabolic syndrome. Of interest is the fact that methylation at each of these sites was associated with a more detrimental profile of phenotypes. We are currently employing next-generation sequencing technologies and large-scale methylation arrays to look for genome-wide alterations in DNA methylation that might contribute to metabolic syndrome in a larger cohort of 1,200 individuals.

P13.42
Identification of a new thyrotropin receptor germline mutations in children with congenital hypothyroidism

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The TSH receptor gene (*TSHR*) encodes a transmembrane receptor , present on the surface of follicular cells which mediates the effects of TSH secreted by the anterior pituitary. Is critical for the development and function of the thyroid gland. It belongs to a subfamily of heptahelical G protein coupled receptors that have a common structure consisting of seven transmembrane segments, three extracellular and three intracellular loops, an extracellular amino terminal domain, and an intracytoplasmic carboxyl terminal tail.

Congenital hypothyroidism (CH) affects 1 in 3000 - 4000 newborns. In Bashkortostan the incidence is 1 in 3800 births. We examined the frequency of *TSHR* mutations among patients with permanent primary congenital hypothyroidism and in the general population of Republic Bashkortostan (Russia).

In total, 82 children aged 8 months to 17.2 years with CH diagnosed by neonatal screening were investigated. All patients had markedly elevated TSH levels concomitant with low peripheral thyroid hormone levels. A reduced thyroid volume or absence of the thyroid gland was determined by ultrasonography or/and scintigraphy. This constellation of clinical findings warranted the diagnosis of congenital hypothyroidism.

We found 4 patients with moderate to severe CH who had monoallelic mutations in TSHR. Observed mutations included one previously characterized mutation (R450H) and three uncharacterized mutations (L653F, L440I, and R531W).

The results demonstrate that mutations in the *TSHR* gene can be responsible for clinical manifestation of congenital hypothyroidism in a relatively small subset of children. So, additional investigations are needed for conformation of pathogenecy for this novel sequence variations.

P13.43
MicroRNA Expression Profiling in Patients with Tyrosinemia Type I

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Tyrosinemia type I is a tyrosine catabolism disease causing tyrosine accumulation resulting from deficiency of the enzyme fumarylacetoacetate hydrolase (FAH), due to mutations in the *FAH* gene. It usually presents with severe liver involvement in young infants. MicroRNAs are small regulatory RNAs that function in post-transcriptional gene expression. miRNA deregulation was observed in the variety of pathologic conditions but their role in the metabolic diseases are not known yet.

We have studied 20 patients with clinical features of failure to thrive, hepatomegaly, liver failure, proximal renal tubular dysfunction, and tyrosinemia which are classical phenotypes of Tyrosinemia Type I. All patients showed either very low or deficient FAH enzyme activity. In order to identify possible miRNAs targeting *FAH* gene transcripts, microRNA expression profiling is performed in Agilent microarray platform representing 723 human and 76 human viral miRNAs in the fibroblasts of these patients. MicroRNA expressions were compared in the fibroblasts, leukocytes and sera of the Tyrosinemia patients. Computational algorithms are also used for prediction of putative mRNA-miRNA interactions. Among them, hsa-miR-768-3p, hsa-miR-513a-5p, and hsa-miR-525-5p are most probable microRNAs targeting the non-conserved sites on FAH mRNA. Verifications of alterations of the expression levels of the selected miRNAs are in progress.

Our data might suggest an active role for miRNAs in the tyrosine catabolism pathway and characterizing miRNA profile in the tyrosinemia patients might help to delineate microRNAs having distinctive role in the disease pathogenesis and also promising targets for future therapeutic studies.

P13.44
Analysis of *UDP 1A9* gene in Polish population using pyrosequencing

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Interindividual diversity in anaesthetic drug metabolism is a major cause of adverse effects, in most cases correlated with the genetic defect in the biotransformation pathway enzymes or receptors. One of the widely used anaesthetics is propofol. Interindividual variability in propofol pharmacokinetics and pharmacodynamics may influence on the anaesthetic function of this drug. Over 99% of this anaesthetic is biotransformated in the liver to several metabolites. The major pathway of metabolism is glucuronidation, catalysed by the microsomal enzyme UDP-glucuronosyltransferase, particularly the isozyme 1A9 (UGT1A9), which belong to the superfamily of enzymes

encoded by the UGT1A gene complex on chromosome 2q37. Several polymorphisms and mutations in this gene have been described. The three crucial of UGT1A9 alleles, UGT1A9 *3 (p.Met33Thr), *4 (p.Tyr242X), *5 (p.Asp256Asn) are associated with decreased or absence of enzyme activity, what determine increased risk of toxic effect. The aim of our study was to optimize a genotyping method to identify the most important in propofol toxicity UGT1A9 variants, and to analyze the frequency of them in Polish population. 100 Polish patients under propofol anaesthesia were examined. The strategy used to identify polymorphisms in the UGT1A9 gene was nested PCR amplification of exon 1 fragments including codon 33, 242 and 256, followed by pyrosequencing. We found a UGT1A9*3 allele frequency of 5%, however the UGT1A9*4 and *5 alleles was not identified, while in others European, Asian and Afro-American populations this variants have been detected.

P13.45
Dysregulation of epicardial, pericardial and subcutaneous adipokines in obesity and coronary artery disease

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Epicardial adipose tissue (EAT), pericardial adipose tissue (PAT) and subcutaneous adipose tissue (SAT) are mediators of obesity and may also be involved in the pathogenesis of coronary artery disease (CAD). We aimed to investigate the association of EAT, PAT; as intra-thoracic visceral fat and SAT with obesity and CAD by the gene and protein expression analysis of related adipokines.

In patients with CAD(n=32) and without CAD(control group) (n=27) subdivided by body mass index of <or=27 and >27kg/m^2, the gene expression of seventeen adipokines were studied by qRT-PCR and the serum level of the most abundant adipokines were analyzed by ELISA.

Gene expression analysis revealed the aberrant mRNA levels of angiotensin,FABP-4,CD68, leptin (LEP),leptin receptor(LEPR),VEGF,TNF-α,Il-6,Il-18,STAMP-2 in EAT,PAT and SAT of patients with CAD and patients with CAD and obesity while the expression of RBP-4 and adiponectin were higher in controls.11β-HSD1 expression was increased in patients with CAD and obesity in EAT,PAT and SAT. In PAT of patients with CAD and patients with CAD and obesity,the expression of angiotensin2 receptor,PAI-1 and C3 were higher than EAT and SAT.The expression of glucocoticoid receptor(GR) was higher in PAT and SAT of patients with CAD and obesity.Leptin serum level was significantly correlated with leptin gene expression in PAT (r=0.539;p=0.01),abdominal fat volume(r=0.484;p=0.03) and subcutaneous fat volume(r=0.605;p=0.005)of patients with CAD.A strong correlation was determined between 11β-HSD1 and LEPR,GR andCD68 genes in EAT,PAT and SAT(p<0.05,respectively).

EAT,PAT and SAT adipokines exhibit a dysregulated mRNA profile in both obesity and CAD.

P13.46
Altered expression of genes associated with atherosclerosis in peripheral blood of subjects with hypertension and subjects with type 2 diabetes mellitus.

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The aim was to identify atherosclerosis related genes specifically associated with type 2 diabetes and with hypertension.

The study includes 36 subjects (23 males and 13 females), divided in 4 age-matched groups: 9 subjects with type 2 diabetes mellitus and hypertension (group 1), 9 subjects with type 2 diabetes mellitus without hypertension (group 2), 9 subjects with hypertension without diabetes (group 3) and 9 healthy subjects as a control group.

Gene expression analysis was performed by Human Atherosclerosis RT^2 Profiler PCR Array. It includes 84 genes related to the formation of atherosclerotic plaques, 5 housekeeping genes and 3 control RNA fragments. Statistically significant up- or down-regulation is accepted as at least 4-fold difference in expression as compared to the control group.

Altered expression of 27 genes was observed in all studied groups as compared to the control group - 23 up-regulated, 4 down-regulated. It was accepted as non-specific.

In subjects with diabetes mellitus, 10 genes matched the criteria for increased expression: APOE, BAX, BCL2L1, LDLR, MMP3, MSR1, NFKB1, PDGFB, SPP1 and TGFB2. No genes with decreased expression were found.

In subjects with hypertension increased expression was found for FAS. Decreased expression in hypertensive subjects was observed for SERPINB2.

Conclusions: Subjects with hypertension and type 2 diabetes mellitus appear to present with non-specific changes in the expression of some atherosclerosis-associated genes in peripheral blood.

The presence of type 2 diabetes increases the expression of 10 genes. The presence of hypertension increases the expression of FAS and decreases the expression of SERPINB2.

P13.47
Enteropeptidase: a gene associated with a starvation human phenotype as a novel target for the treatment obesity and type II diabetes

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Many obesity related genes have been proposed as targets for the treatment of obesity. However, these obesity genes did not provide efficient drug therapy for obesity treatment. This is mainly due to the redundancy of the biochemical pathway involved in obesity and the lack of specificity of the gene targets. It is therefore a challenge to identify crucial gene(s) targets involved in energy metabolism associated with “lean or starvation phenotype”.

Congenital Enteropeptidase defficiency is an extremly rare pathology which answer to all these criteria. Enteropeptidase catalyzes the conversion of inactive trypsinogen into active trypsin *via* the cleavage of the acidic propeptide from trypsinogen

We have generated knock out transgenic mice for enteropeptidase which shows the same phenotype like in human. These data and in vivo preclinical data using per os small molecule for long term treatment (9 weeks) will be presented.

P13.48
Lack of association of a fatty acid amide hydrolase (FAAH) gene variant to weight loss in a one-year intervention for obese children and adolescents (Obeldicks)

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Introduction: Recently, it was shown that obese carriers of the A allele of SNP rs324420 in FAAH lost more weight and improved associated phenotypes better than non-carriers during an intervention. We tried to replicate this finding in obese children and adolescents undergoing a one year lifestyle intervention (Obeldicks progam).

Subjects and methods: 453 overweight and obese children and adolescents (10.8 ± 2.6 years, BMI-SDS 2.4 ± 0.5; 55% girls) were genotyped for rs324420 (C/A) by restriction fragment length polymorphism (RFLP) analysis. Participants received a balanced diet, containing 55 En% carbohydrates, 30 En% fat and 15 En% proteins. Moreover, they took part in an exercise therapy once a week. Blood was

taken at baseline and after one year of intervention. Anthropometric (height, weight, BMI and BMI-SDS) and plasma parameters (total cholesterol, LDL-cholesterol, HDL-cholesterol, triacylglycerides, glucose, insulin and HOMA) as well as blood pressure were measured.
Results: Both BMI and BMI-SDS improved significantly among all subjects. The mean systolic blood pressure was also lowered and concentrations of HDL-cholesterol increased significantly. However, none of the measured parameters were associated with FAAH rs324420 genotypes.
Conclusion: We did not detect association of FAAH genotypes with weight reduction in overweight and obese children and adolescents. Hence, the previous finding in adults could not be confirmed. As the length (1 year to 3 month) and the set up (hypo caloric diet in adults versus physical activity plus balanced meals) of the interventions differed, these parameters might have influenced the different results.
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P13.49
Maturity Onset Diabetes of the Young (MODY) in an Italian family associated to a mutation disrupting the HNF-1 binding site at P2 promoter of HNF4A gene

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Backgroud: Maturity-onset diabetes of the young (MODY) is a heterogeneous group of disorders characterized by early onset of non-insulin-dependent diabetes and autosomal dominant inheritance. At least 9 MODY-causing genes have been identified.
Aims and Methods: we aimed to investigate the promoter P2 of HNF4A gene in 97 families with suspected MODY, that were negative for mutation in coding sequence of the most common MODY genes GCK, HNF1A and HNF4A. The proximal P2 promoter was screened from -365 using dHPLC and sequencing.
Results: the -169 C>T was detected in a female patient (diabetes at age 8) and her brother (diabetes at age 11), both displaying phenotype of HNF4A/MODY1. The variant was absent in 214 normal chromosomes. The substitution lies within a known binding site for HNF-1alpha in a conserved nucleotide position. Evidence for the pathogenicity of this mutation was obtained in transient plasmid transfection assays with mutated and wild-type P2 promoter. Luciferase activity assay revealed a markedly decreased activity of the mutant allele compared to the wild type in cancer cell lines and in freshly isolated rat primary pancreatic islets.
Conclusion: our results indicate that the -169C>T mutation at the HNF4A P2 promoter gene plays a causative role in MODY, due to an impairment of the HNF-1alpha-dependent activation of HNF-4alpha in adult beta-cell. This is the first time that the P2 promoter activity is investigated in freshly isolated primary rat pancreatic islets. Our finding highlights the need to include the P2 promoter in the molecular screening of HNF4A gene.

P13.50
Mutation screening of *PAH* gene in Slovak Pku patients using high-resolution melting

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Phenylketonuria (PKU) is an autosomal recessive inherited disorder arising from a deficiency of phenylalanine hydroxylase (PAH), which catalyses the essential conversion of phenylalanine (Phe) to tyrosine (Tyr). In the majority of cases, PKU is caused by mutations in the *PAH* gene, and it presents with different phenotypes which are classified according to Phe tolerance. More than 500 mutations have been described world-wide and the PAH enzyme has been fully characterized. The incidence of the disease in the Slovak population had been estimated to be 1:10 000 newborn. In our previous studies complete mutation spectrum among Slovak PKU patients has been characterized. So far we are able to detect approximately 95% of mutant alleles with direct sequencing and MLPA analysis. In this work we present high-resolution melting (HRM) as an effective, sensitive and cheap method for mutation screening of the *PAH* gene. PCR conditions for all 13 exons of the gene were optimized for HRM analysis. Melt profiles were acquired on a 96 well LightScanner instrument (Idaho Technology) and melt data were normalized and temperature shifted. All of the known mutations within amplicons were detected by shifted melting curves, what indicates high sensitivity of the method. However, some polymorphisms in the ends of amplicons were not detected. Details of our work will be presented on poster.

P13.51
Mutational analysis of phenylalanine hydroxylase gene in Slovenian patients with phenylketonuria: five novel mutations

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Phenylkatonuria (PKU, OMIM 261600) is an autosomal recessive metabolic disorder arising from the deficiency of hepatic enzyme phenylalanine hydroxylase (PAH). In the majority of all cases PKU is caused by missense mutations in the PAH gene, which maps to chromosome 12, region 12q23.2.
The average birth incidence of PKU in Slovenia is approximately one in 8000. Currently, Slovenian national PKU register contains around 130 patients, 107 of them were included into our mutational analysis of PAH gene. The entire coding region of the PAH gene spanning 13 exons was PCR amplified and analyzed with denaturing high-performance liquid chromatography (dHPLC). On all the identified PCR fragments we subsequently performed an automated sequencing analysis.
The cumulative mutation detection rate in this group of patients was 95%. We have identified 32 different disease-causing mutations, five of them novel: p.L15GfsX24, p.V45A, p.L62P, p.R157S and c.56_60delACAGG. Twelve patients were found to be homozygous (two for the p.R158Q mutation and the rest for the p.R408W), all the other patients were compound heterozygotes. The single most frequent mutation in Slovenian PKU population is p.R408W in exon 12 (34%), which is in concordance with previous European and regional studies of PAH gene. 30-40 patients are likely to be BH4 responsive.
dHPLC proved to be a fast and sensitive method for mutational screening of the PAH gene. In combination with sequencing method, it represents a reliable and cost-effective diagnostic tool for detection and identification of unknown molecular defects in patients with PKU.

P13.52
Mutation analysis of phenylketonuria (PKU) in south western of Iran

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Introduction: Deficiency in hepatic phenylalanine hydroxylase (PAH) causes phenylketnouria that is an autosomal recessive and heterogeneous metabolic disorder. Molecular basis of this most prevalent disorder is frequent mutations in phenylalanine hydroxylase gene. PAH require tetrahydrobiopterin as a cofactor for conversion of phenylalanine to tyrosine. Loss of PAH activity results in accumulation of phenylalanine in tissues and biological fluids that cause the formation of secondary neurotoxic metabolites. To date more than 600 mutations in the *PAH* gene have been identified and submitted to the *PAH* and HGMD databases. The prevalence of phenylketonuria In Europe or among the general Caucasian populations is about 1 in 10,000. In Iran the incidence of PKU might be about 1.6 in 10,000.
Methods: This study enrolled 40 Iranian southwestern residents. DNA extraction from peripheral blood leukocytes performed by Diatom DNA prep 100 kit. Using manually designed primers, PCR amplification accomplished for the exons 6, 7, 10 and 11 of *PAH* gene. Then we used direct sequencing of amplified fragments for identification of mutations. Analysis of DNA sequences performed by Mutation Surveyor software.
Results: In this study, we observed eleven mutations, I224T, S231P, R176X, c.592_613del22, R243X, R252W, R261Q, Y356X, IVS10-11G>A, IVS11+1G>C and Q375R. The Q375R is a new mutation in exon 11. V245V, Q232Q and L385L were three detected silent mutations with high frequency.

Discussion: Khuzestan population comprises a heterogeneous ethnic group in southwestern of Iran. With mutation detection rate equal to 46.25%, 40% of patients were homozygous and 10% were heterozygous that indicate high consanguinity.

P13.53
Long-chain-3-hydroxyacil-CoA dehydrogenase deficiency - the most frequent fatty acid oxidation disorder in Latvia: 7 cases

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Background: Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency is one of the defects of mitochondrial β-oxidation and the only one of all fatty acid disorders diagnosed in Latvia during the last 13 years.
Objectives: To characterize clinical, biochemical and molecular data in 7 patients with LCHAD deficiency, found by selective screening in Latvia.
Methods: Retrospective analysis of clinical and laboratory findings in our patients.
Results: Five patients presented during early infancy (2.5 months - 6.5 months) with vomiting, poor feeding, lethargy, hepatopathy and nonketotic hypoglycemia. Three of them developed coma and seizures. One patient presented at the age of 21 months with hypoglycemic coma and seizures, psychomotor retardation and one patient was diagnosed before clinical symptoms. Two patients were born preterm with severe intrauterine growth retardation. During metabolic crisis all patients had typical acylcarnitine profile, increased level of 3 - hydroxydicarboxylic acids in urine, elevated liver enzymes, moderate lactic acidemia and hyperammonemia. All patients had homozygous mutation 1528G>C in *HADHA* gene. Acute hepatopathy during prenancy was reported in three cases. After infancy progressive retinopathy occurred in three patients, two patients had episodes of severe muscle pain and rhabdomyolisis during intensive physical activities or illnesses.
One patient died at the age of 6.5 months before diagnosis was made, other died at the age of 10 years during acute gastroenterocolitis.
Conclusion: LCHAD deficiency is frequent fatty acid oxidation disorder in Latvian population. Newborn screening would be important for early detection of all patients.

P13.54
Neurodegeneration in Cobalamin A Deficiency

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Cobalamin (Cbl) A deficiency is rare cause of Cbl-responsive methylmalonic academia. It results from defect in mitochondrial Cbl reductase, encoded by *MMAA*. Treatment with B12 reduces the amount of MMA. Liver or stem cell transplantation has been applied in some cases. Despite therapy patients suffer progressive neurodegenerative course resulting in significant disability. The metabolic defect underlying nerve degeneration is unknown but some authors propose synergistic toxic effect of methylcitrate, malonic acid, propionyl-CoA on respiratory chain and tricarboxylic acid cycle resulting in brain damage.
An 11year old boy born to consanguineous marriage developed normally until 3 years of age when he suffered a metabolic stroke during an intercurrent viral illness. Brain MRI showed bilateral stroke-like lesions in the globus pallidus. He was diagnosed with Cbl A deficiency based on elevated MMA responsive to vitamin B12 challenge and was found to have homozygous c.1034delT frameshift mutation in *MMAA*. Despite cyan-cbl carnitine and metronidazole therapies, protein restriction and stem cell transplant within 1.5 years after his acute presentation he lost ability to walk and his speech output decreased due to severe dystonia that started in left upper extremity but progressively generalized. Dystonia improved with deep brain stimulation at 11 years.
Standard treatment of Cbl-responsive MMA has improved survival but has not modified neurodevelopmental outcome. Stem cell transplantation did not alter disabling neurological deterioration in our patient but DBS improved dystonia. The mechanism underlying progressive brain damage is uncertain but new therapies targeting proposed secondary mitochondrial respiratory chain dysfunction and antioxidants should be investigated.

P13.55
Mutation in the *OTC* promoter may cause symptoms of ornithine carbamoyltransferase deficiency

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Ornithine carbamoyltransferase deficiency (OTCD; OMIM311250) is the most common inherited defect of the urea cycle. Mutation analysis of the coding regions of *OTC*, the enzyme catalyzing the synthesis of citrulline in the liver, fails in 20-25% of patients manifesting OTCD. In such cases, the disease may be caused by gross gene rearrangements or defects in regulatory regions of the gene.
In our study we examined the 5'UTR, identified and functionally characterized *OTC* regulatory elements and estimated the pathogenicity of a promoter variation c.-366A>G found in a female patient with mild symptoms of OTCD.
Three alternative transcription start sites located 95, 119 and 169 bp upstream the iniciation codon were determined within the *OTC* 5' UTR. The human *OTC* promoter and enhancer elements were predicted from the homology with rat and mouse and functional functionaly characterized using dual lucisferase reporter assay. Functional studies revealed a moderate transcriptional activity of the promoter in both liver and kidney derived cells. Significantly higher and liver specific *OTC* expression was observed when promoter interacted with the distal enhancer. The promoter variation c.-366A>G did not affect the function of the promoter alone but it disrupted the interaction of the promoter with the enhancer, which was observed as 50% decrease of the transcriptional activity.
Our data indicate that full transcriptional activity of human *OTC* promoter depends on an upstream enhancer. The promoter - enhancer interaction contribute to tissue specific expression of *OTC* in the liver and the disruption can lead to the manifestation of OTCD.
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P13.56
'Full-blown' Lowe syndrome without congenital cataracts in a patient with an *OCRL* p.Gln199X mutation and a silent variant, p.Arg35Arg.

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The oculo-cerebrorenal syndrome of Lowe is an X-linked recessive disorder characterized by the triad of congenital cataracts, mental retardation and incomplete renal Fanconi syndrome. Additionally, various neurological signs may be present such as developmental delay, seizures, hypotonia, areflexia or behavioural disturbances. Patients show progressive growth retardation and may develop a debilitating arthropathy. Although phenotype might vary, congenital cataracts have been assumed the first and obligatory sign.
We report a 13 year old patient showing the complete phenotypic spectrum of Lowe syndrome who was not classified as having the disease due to the absence of any ocular involvement. *OCRL* gene analysis revealed a Gln199X (c.760T; according to ENST00000371113) mutation and a silent variant (c.G270A; p.Arg35Arg) in the patient. We suggested that the synonymous substitution might effect correct splicing, however an *in vitro* model revealed only normally spliced transcripts. Moreover, RNA obtained from the patients' EBV transformed lymphocytes showed presence of both transcripts due to alternative splicing of exon 18a.
Taken together, the silent variant provided no clue for the milder phenotype in the context of a classic Lowe mutation (p.Gln199X). This finding extends the phenotypic spectrum caused by *OCRL* mutations and illustrates that even in the absence of congenital cataracts Lowe syndrome has to be assumed, if all other features are present.

P13.57**

Riboflavin responsive OXPHOS complex I deficiency caused by defective ACAD9: new function for an old gene

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Mitochondrial complex I deficiency is the most common OXPHOS defect. Mutations have been detected in mitochondrial and nuclear genes, but many patients remain unresolved and new genes are likely involved. In a consanguineous family, patients presented since early childhood with easy fatigability, exercise intolerance and lactic acidosis in blood. In muscle, subsarcolemmal mitochondrial proliferation and a severe complex I deficiency were observed. Exercise intolerance and complex I activity improved by supplementation with a high dosage of riboflavin. Homozygosity mapping revealed a candidate region on chromosome three containing six mitochondria-related genes. Four genes were screened for mutations and a homozygous substitution was identified in ACAD9 (c.1594C>T), changing the highly conserved arginine-532 into tryptophan. This mutation was absent in 188 ethnically matched controls. Protein modelling suggested a functional effect due to loss of a stabilizing hydrogen bond in an α-helix and a local flexibility change. To test whether the ACAD9 mutation caused the complex I deficiency, we transduced fibroblasts of patients with wild type and mutant ACAD9. Wild type ACAD9, but not mutant ACAD9, restored complex I activity. An unrelated patient with the same phenotype was compound heterozygous for c.380G>A and c.1405C>T, changing arginine-127 into glutamine and arginine-469 into tryptophan respectively. These amino acids were highly conserved and the substitutions were not present in controls, making them very likely pathogenic. Our data support a new function for ACAD9 in complex I function, making this gene an important new candidate for patients with complex I deficiency, which could be improved by riboflavin treatment.

P13.58

The E670G SNP in the PCSK9 gene in polish population with acute myocardial infarction

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The SNP at E670G within exon 12 is one of the main genetic alteration determining plasma low density lipoprotein cholesterol (LDL-C) level in human.

The aim of the study was to determine the incidence of the E670G SNP polymorphism in PCSK9 in polish population with acute myocardial infarction and investigate the contributions of this genetic variant to differences in plasma levels of LDL-C.

The investigated group consisted of 72 individuals with a mean age of 62 years, and with a mean plasma LDL-C level of 137 mg/dl. For the determination of the frequency of the E670G polymorphism a genomic DNA was amplified by standard PCR with primers specific for 12 exon. Each product was sequenced on an CEQ 8000 (Beckman Coulter).

Analysis of exon 12 sequence shows homozygous AA genotype in 62 individuals and heterozygous AG in 10 subjects. Additionally, the G allele of the E670G SNP in the PCSK9 gene was present at an increased frequency in participating men in comparison to women. LDL-C level was non-significantly higher in those with the AG in comparison to the AA genotypes (mean 165 mg/dl and 133 mg/dl respectively, p=0,09).

Neverthelater identification of another PCSK9 gene variants affecting LDL receptors density on cell surface and LDL-C level could provide a wider insight into a pathogenesis of atherosclerosis.

P13.59

Molecular diagnostic in patients with hepatocerebral mitochondrial encephalomyopathies

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We present the results of molecular genetic investigations in a group of 13 children with Alpers syndrome (A.S.) and mitochondrial hepatocerebral syndrome (MHS). We performed sequence analysis of POLG in patients with strong suggestion of AS. In one AS patient only one mutant allele revealed. Two infant patients with MHS phenotype also had mutations in POLG gene (in heterozygous state). Depletion of mtDNA was detected in blood only in patients with DGUOK and MPV17 genes mutations. Anatomopathological issue in the patient with POLG mutations (A467T/ G268A) showed necrosis lesions in basal ganglia and brain stem (typical for Leigh syndrome phenotype). Our data confirm that test for POLG gene mutations is very important in patients with mitochondrial hepatopathy and not only in AS phenotype. Complicating factors are search for a second mutant allele and helpless use of blood DNA for mtDNA depletion detection.

phenotypes and genotypes in patients with AS and MHS

phenotype	mutant gene	1st mutant allele	2nd mutant allele	mtDNA depletion in blood
AS	POLG	p.W748S	p.T885S	N/A
AS	POLG	p.W748S	p.L311P	N/A
AS	POLG	p.A467T	p.G268A	N/A
AS	POLG	p.W748S	p.G848S	N/A
AS	POLG	p.A467T	?	-
AS	POLG	p.A467T	p.G268A	-
AS	POLG	p.W748S	c.3632del12	-
AS	POLG	p.R627Q	R807H	-
AS	POLG	p.W748S	p.W748S	-
MHS	POLG	p.G737R	?	-
MHS	POLG	p.W748S	?	-
MHS	DGUOK	p.R105X	p.R105X	+
MHS	MPV17	p.R41W	c.185delT	+

P13.60

Assessment of three various screening tests for easy detection/ exclusion of common SCO2 mutation among patients with cytochrome c oxidase deficiency

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SCO2 is one of the ancillary proteins necessary for correct assembling and functioning of cytochrome c oxidase (COX). It is involved in the transport and incorporation of the copper ions to the CuA enzymatic site on COXII subunit of the enzyme. Mutations in the *SCO2* gene (22q13) lead to serious damage of the protein resulting in severe COX deficiency observed mainly in muscles, heart and brain. The common substitution is g.1541G>A (p.E140K), which was identified at least on one allele in all so far reported patients.

The aim of this study was to optimize conditions to determine the best method for screening of g.1541G>A mutation in COX-deficient patients.

In the study were selected three molecular techniques; two - based

on Real Time equipment (TaqMan probes and HRM), and one - on gel electrophoresis (SSCP). In HRM method, melting curve analysis is used for identification of mutations. Genotyping with Taqman's probe enables identification of specific mutation. SSCP identifies changed samples by comparison their electrophoretic mobility to control samples in polyacrylamide gel.
Preliminary studies provided reliable results from all mentioned methods.
Advantages and disadvantages of every method will be evaluated, which will allow to choose the best of them.
Determination of the rapid genetic screening test for COX deficiency caused by mutations in the *SCO2* gene let confirm a final diagnosis and will enable establishment of treatment decisions in affected children.
The study was supported by the project no. N N407 115440.

P13.61
Spastic paraplegia due to CYP7B1 mutations (SPG5) : what can we learn about 27-hydroxycholesterol metabolism?

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Hereditary spastic paraplegias (HSP) constitute a clinically and genetically heterogeneous group characterized by a progressive 'dying back' degeneration of the cortico-spinal tracts. Mutations in CYP7B1 were identified in SPG5 families presenting with a pure form of HSP (Tsaousidou et al, 2008; Goizet et al, 2009). CYP7B1 encodes the cytochrome P450 7α-hydroxylase implicated in cholesterol metabolism, and plays a role in the alternate/acidic pathway for primary bile acid production. Increased levels of oxysterols, 27- and 25-hydroxycholesterol were recently identified in the plasma of 4 SPG5 patients (Schüle et al, 2010). Besides the potential consequences on hepatobiliary functions of altered bile acids synthesis in SPG5, in vitro studies suggest that 27-hydroxycholesterol - an endogenous selective estrogen receptor modulator - may decrease bone mineral density, inhibit the cardiovascular protective effects of estrogens and increase oxidative stress in retinal pigment epithelial cells. Therefore, we investigated 9 SPG5 patients from 6 families in order (i) to confirm that plasma oxysterols can be used as biomarkers in HSP due to CYP7B1 mutations, and (ii) to explore the non-neurological manifestations that may result from the altered oxysterols metabolism in SPG5 patients - i.e. bone homeostasis, hepatobiliary and cardiovascular functions, retinal trophicity. Preliminary data suggest that the elevation of plasma 27-and 25-hydroxycholesterol in our SPG5 cohort is associated with systemic complications, and in particular reduced bone density. Better delineating the non-neurological manifestations that may occur in SPG5 should also shed light on the best therapeutic approaches aiming at lowering 27-hydroxycholesterol levels.

P13.62
Molecular characterization in Japanese patients with classical xanthinuria.

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Classical xanthinuria (OMIM #278300) is an inherited metabolic disorder caused by a deficiency of xanthine dehydrogenase (XDH), resulting in hypouricemia. Classical xanthinuria frequently presents with renal calculi and occasionally leads to renal failure. Classical xanthinuria is classified into two categories. Classical xanthinuria type I lacks only XDH activity by XDH gene mutations, while the type II results from dual deficiency of XDH and aldehyde oxidase due to mutations in the molybdenum cofactor sulfurase (MOCOS) gene. Classical xanthinuria is easily differentiated by means of the allopurinol loading test. As allopurinol is converted to oxypurinol by both XDH and aldehyde oxidase, oxypurinol is detected in urine and serum of subjects with classical xanthinuria type I upon administering allopurinol.
We studied fifteen Japanese hypouricemic subjects with classical xanthinuria from fourteen different families at Jikei University School of Medicine. We report the spectrum of mutations identified to data. Eleven subjects were xanthinuria type I, while three subjects were xanthinuria type II. We could not verify a causative mutation of XDH and MOCOS genes in one subject with xanthinuria. The percentage of xanthinuria type I in xanthinuria was 78.6 %. In xanthinuria type I, mutation C2567Del was identified most in eight alleles out of thirty alleles, while C682T, leading to R228Ter, was identified in five alleles out of thirty alleles. In xanthinuria type II, most frequent C1255T, leading to R419Ter, is identified in four alleles out of six alleles. Any mutations did not dominate in XDH mutations of xanthinuria type I.

J13.01
Glycogen-Storage Disease Type I b - Case Report

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Glycogen Storage Disease type 1b due to glucose-6-phosphatase deficiency, is a type of glycogenosis due to glucose-6-phosphate deficiency. Glycogen-storage disease type Ib is autosomal recessive genetic trait caused by mutations at loci 11q23. The incidence was estimated at 1: 100,000.
This male patient was first-born child. This was controlled and non-complicated pregnancy. Parents are not consanguineous. The baby was born on term. Growth parameters were weight=3750 g; length= 54 cm, head circumference 34 cm. Apgar score was 8/9. The proband developed hypoglycemia and sepsis very soon after birth. He developed hepatomegaly and splenomegaly confirmed with ultrasound. His growth and development were delayed and he had repeated episodes of pyogenic infection.
Physical examination in the second year of life revealed growth retardation, microcephaly, rounded "doll's face" appearance, abnormal earlobes, divergent strabismus, gothic palate, long eyelashes, small mouth, thin lips, hypertelorism and abnormal sole creases. He had a distended abdomen with an enlarged liver over 7 cm below the costal margin and enlarged spleen below 5 cm the costal margin. He had cryptorchismus. His motor and mental development were retarded, he had hypotonia. Laboratory findings revealed neutropenia, hypoglycemia, hyperlactatemia, hypertriglyceridemia, hyperuricemia.
Regular administration of G-CSF improved his health and decreased his hospital stay.
Diagnosis of GSD Ib was confirmed after detection of homozygous mutation c.1042_1043delCT on his SLC37A4 gene.

J13.02
Glut1 deficiency syndrome: clinical, biological and therapeutic aspects

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The medical and personal history of a 5 year old boy in observation since he was 2 proves for the good communication between family and specialists. Glucose transporter type 1 deficiency syndrome (Glut1-DS) characterized by infantile seizures refractory to anticonvulsants, is associated with head growth, mental and motor development delays, spasticity, ataxia, dysarthria, opsoclonus and other paroxysmal neurologic symptoms, often prior to meals. Andrei had been considered normal at birth. Birth weight and Apgar scores were normal. His first seizure occurred at age 3 as myoclonic jerking of shoulders and arms, nodding of the head, eyes rolling, hypotonia, impaired consciousness. Seizures prevailed every morning. The delay was mainly in language and motor coordination. MRI showed moderate frontal atrophy. Fasting EEG revealed mild to moderate slowing of background activity and multifocal and generalized high-amplitude irregular spikes and spike-and-waves; most of discharges were clinically silent, occasionally they were accompanied by myoclonic jerks of shoulders and arms or nodding of the head. EEG recording 1 h after breakfast showed disappearance or marked reduction of epileptic activity. Specific investigations confirming Glut-1DS like erythrocyte glucose uptake and mutation analysis of GLUT-1 are time consuming and expensive.

Starting from mother observations we performed pre- and postprandial EEG recordings in a boy with seizures aggravated by fasting and ameliorated by i.v. Glucose administration. This simple examination provides a powerful tool to identify patients with possible Glut-1 DS.

J13.03
Metabolic biomarkers in Children with Autism Spectrum Disorders

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Autism is a complex neurodevelopmental disorder and thought to be influenced by genetic factors. the prevalence of inherited metabolic disorders are very high in Iranian (1/1000 birth) ,and their symptoms may miss by wrong diagnosis .
Objective: Evaluate plasma concentrations of abnormal metabolites of amino acids & acylcarnitine by HPLC chromatography (blood - urine) and MS/MS methodes in children diagnosed with autism Spectrum Disorders.
Criteria:
Inclusion :
- Diagnosis of autism spectrum disorder based on clinical evaluation
- Age ≥ 24 months -
- gender: both
Exclusion :
Acquired developmental disability or cerebral palsy.
children with deafness, dysmorphism, congenital malformations, short stature, birth defects.
Method : Seventy two autistic children, 60 boys and 12 girl, between the ages of 2-7 years (2008-2010), and satisfying the DSM-III-R criteria (American Psychiatric Association, 1987) for the diagnosis of autism, were selected to participate in this study.
Plasma concentrations of aminoacids & acytylcarnitine were measured in 72 children with autism and in 80 control children.
• Results:
male:female ratio 5:1
9 patient had positive metabolic evaluation (confirmed by urine organic acids , molecular genetics tests
18% positive family history and in 70% consanguinity marriage.
• The most frequent diagnoses were fatty acid oxidation disorders and organic acid disorders
Conclusions: These results are similar to those of the comparison studies, although the overall prevalence IEM is higher in this study. This is probably due to the effects of ethnicity and consanguinity and increasing ascertainment.

J13.04
Cystic fibrosis and bronchic responsivity

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Background: The aim: the evaluation of bronhic responsivity (BR) in cystic fibrosis (CF) patients. Methods: Patients with CF (11 cases) and patients with CF and bronchial asthma (4 cases), patients with CF and allergic bronchopulmonary aspergillosis (ABPA) - 2 cases . Spiromery tests used: metacholin (if FEV1≥ 80 %) and β2 agonist (if FEV1≤ 80 %) of predicted value. Tests were considered positive: decrease of FEV1 with 20 % comparative to the control value ($PC_{20\%}$ FEV1) for methacoline test, respectively increase of FEV1 ≥ 15 %(in beta mimetic). In patients with asthma we effectuated prick tests and specific IgE antibody for *Aspergillus fumigatus*. Results: In the group with CF, 7 patients presented mixed moderate or severe ventilator dysfunction; 4 of them had positive BD test and 3 had negative test. Four patients of the same group had normal basal ventilometric parameters. Two of them presented positive test with metacholin for medium BR. In the group of the CF and asthma two presented moderate BR, one had normal ventilometric indexes and one presented mild obstructive ventilator dysfunction with positive bronchodilatator (BD) test. In these patients prick tests and the specific IgE antibody were negative. ABPA was confirmed through cultures and IgE specific. The evolution of these cases was towards severe mixed ventilator dysfunction with positive BD test. Conclusion: Bronchial hyperresponsivity is a common phenomenon in CF, with variations from one patient to another, increases in time and was present at all clinical situations from our study.

J13.05
Absence of ambiguous genitalia in a girl with salt wasting form of 21-hydroxylase deficiency

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Introduction: Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders, mainly known by causing ambiguous genitalia in girls. It is caused by mutations in cortisol biosynthesis enzymes. 90-95% of the cases are occurred due to mutations in CYP21A2 gene. There is a range of phenotypic variability because of different mutations of the gene.
Method and case presentation: We report here a newborn girl with no ambiguous genitalia but 21 hydroxylase deficiency.
Results: Biochemical assays of the patient showed high level of 17 hydroxyprogesterone. She enrolled to the hospital with no sign of virilization. CYP21A2 gene mutations were determined by direct sequencing.
Discussion: There is a need for detailed examination of newborns for CAH at diagnosis and pediatricians should be aware of the cases with salt wasting crisis with no ambiguous genitalia. Molecular genetic analysis would confirm the mutation for the diagnosis. Genotype-phenotype discrepancy could be interpreted by the effect of modifying factors during pregnancy.

J13.06
Congenital adrenal hyperplasia and mutation detection in Iran

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Background: Congenital adrenal hyperplasia (CAH) accounts for one of the important causes of ambiguous genitalia in girls. 90-95% of the causes around the world are due to CYP21A2 gene mutations.
Methods: CYP21A2 gene is examined in patients affected with CAH with allele specific PCR and multiple ligation probe amplification (MLPA) methods. Direct sequencing is performed for those with rare and novel mutations.
Results: To date, deletion and chimers include about 30% of the CYP21A2 gene. IVS2-13A/C > G, Q318X, R356W, I172N, E6 Cluster, 707_714del8 mutations defined the rest of deleterious mutations, respectively.
Conclusion: Congenital adrenal hyperplasia (CAH) is one of the common causes of ambiguous genitalia in Iran. Newborn screening programs should be used since there are lots of consanguineous marriages in the country. Carrier detection and prenatal diagnosis is performed in the country.

J13.07
Familial hypocalciuric hypercalcemia - a case report

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Introduction: Familial Hypocalciuric Hypercalcemia (FHH) is a rare form of asymptomatic hypercalcemia. The vast majority of FHH is caused by autosomal dominant loss-of-function mutations in the gene CASR, which codes for the calcium-sensing receptor (CASR).

Loss-of-function mutations in CASR impair the feedback inhibition of parathyroid hormone secretion in response to a rise in the blood calcium concentration. Thus, higher than normal blood calcium levels are necessary to inhibit release of parathyroid hormone, resulting in hypercalcemia associated with normal or mildly elevated levels of parathyroid hormone. Material: We report the case of a 11 month old female baby, admitted for the closure of a large persistent ductus arteriosus, who was accidentally discovered with a high blood calcium level of 2,99 mmol/l (normal values 2,10 -2,60), ionized calcium 1,38 mmol/l (1,03 - 1,30), normal phosphatemia, having actually no symptoms of hypercalcemia. The child presented moderate hypotrophy and dyspneea due to a large left-to-right shunt. The renalfuntion was normal. The calciuria was decreased 1,25 mmol/24h (1,75 -7,5 mmol/l). Urinary creatinine was low 0,45 mmol/24h (5 - 18). Parathormon levels were moderately increased 108 pg/ml (10 - 70 pg/ml). No genetic testing was possible in that child. The child had an unevenfull closure of ductus arteriosus, and thrive well now. Conclusions: The benign course of the disease require no medication for lowering the calcium levels, nor is parathyroidectomy indicated.

J13.08
Analysis of SMPD1 gene in an Iranian patient affected by Niemann Pick disease type B

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Niemann-Pick disease, an inborn error of sphingomyelin catabolism, results from the deficient activity of the lysosomal hydrolase, acid sphingomyelinase (sphingomyelin phosphodiesterase; E.C.3.1.4.12). Two allelic forms of this autosomal recessive disorder have been delineated based on their distinct phenotypes. Type A Niemann-Pick disease is a severe neurodegenerative disorder of infancy characterized by progressive psychomotor retardation, hepatosplenomegaly, and death by three years of age. In contrast, Type B Niemann-Pick disease is a nonneuronopathic disorder characterized by hepatosplenomegaly, respiratory involvement, and survival into adulthood. The clinical course of patients with Type A disease is quite uniform, whereas the severity among Type B patients is more variable. In fact, some mildly affected Type B patients may survive into the sixth decade of life. Our case was a 6-year-old girl characterized by hepatosplenomegaly, respiratory involvement, hyperlipidemia and thrombocytopenia who affected by Niemann Pick type B. Enzyme activity in peripheral blood lymphocytes for this patient was less than 10% of controls. Whole SMPD1 gene sequencing analysis of this proband depicted a homozygote mutation in codon 36 which changes codon Valine to Alanin in exon 1. For confirmation of the mentioned mutation, we analyzed exon 1 of SMPD1 gene for her parents. Sequencing analysis showed heterozygote state for this alteration

J13.09
MnSOD Val16Ala polymorphism in Romanian patients with Type 1 Diabetes and Retinopathy

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Diabetic retinopathy (DR) is one of the leading causes of blindness and has an unclarified pathogenesis in which genetic predisposition may play a role. Hyperglycemia increases oxidative stress which may trigger the development of DR. SOD2 is a key enzyme which protects cells from oxidative damage. Functional polymorphisms in SOD2 gene may result in predisposition to DR. The aim of this study was to evaluate the possible association between Val16Ala polymorphism (rs4880) in SOD2 gene and DR, in type 1 diabetes.

We enrolled 189 unrelated Caucasian patients with type 1 diabetes divided into a group A - patients with diabetes duration over 15 years without DR (n=70) and group B - patients with DR (n=119). Genotyping was achieved by Taqman. Statistical analysis was performed using DeFineti software and Cochran-Armitage correction was applied.

Genotype spread analysis indicated that ValVal genotype is more frequent in the DR group (22.68%) compared with controls (17.14%). There was a significant deviation from Hardy-Weinberg equilibrium in the control group (X2=8.22,F=0.343). Presence of homozygous 16Val allele seem to increase the risk for DR (OR=1.333), while the 16Ala allele seems to be protective (OR=0.750), but he results are not statistically significant (p=0.55). Corrected OR for both alleles are not reaching statistical significance.

This is the first study of Val16Ala polymorphism in DR from Romanian population. Although this is a small study that suggests there is no association between Val16Ala polymorphism and DR, further studies are necessary in order to clarify the role of SOD2 gene in DR.

J13.10
Clinical, Enzymatic, and Molecular Diagnosis of Mucopolysaccharidoses in Iran, a Five Years Experience

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Mucopolysacchridoses are prevalent in our population, constitute a very important health problem and burden in this community. High rate of consanguineuous marriages, one of the important predisposing factors. They are “Enzymopathies” and the result of homozygous or compound heterozygous mutations in different genes which are responsible to encode specific enzymes inside the cells. Each enzyme catalyzes one of the different metabolic reactions that is necessary to degrade intra-lysosomic glycosaminoglycans. Result of the mutation in a coding gene may cause severe enzyme deficiency, intra-lysosomal accumulation of glycosaminoglycan macromolecules, and a specific phenotype. Typical sequence of symptoms may include progressive neurodevelopmental delay or regression, behaviour abnormality, progressive dysmorphic-course facies, corneal clouding, hearing loss, visceromegaly, skeletal dysplasia, and dysostosis multiplex congenita. We have studied 72 families in the past 5 years, that were referred for evaluation and diagnosis of a dysmorphic, neurodevelopmental regressive disorders with the MPS phenotype. The diagnosis in the index cases (after a detailed clinical, biochemical, radiological, imaging studies) confirmed by enzyme assay and in a few of the cases by molecular analysis.

We also determined to use Enzyme Replacement Therapy in some of our patients with type I, type II, and type VI, and also performed BMT, in a few of them.

The result of this experience on clinical, enzyme assay, and molecular analysis would be presented in the congress.

Keywords: MPSs, Mucopolysaccharidosis, Macromolecular Enzymopathies, Iran Experience

J13.11
Methylmalonic aciduria and Homocystinuria detected by joint NMR and chromatography techniques: A Case Report.

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Methylmalonic aciduria is a genetically heterogeneous metabolic disorder due to abnormal activity of methylmalonyl-CoA mutase.

We report on an 11 months boy, hospitalized for the second time in intensive therapy with recurrent vomiting, dehydration, ketonuria, metabolic acidosis accentuated after high protein intake. The parents are not consanguineous, their older boy died from unidentified similar metabolic conditions, age 10 months.

The blood and urine amino acids were examined by liquid chromatography. The elevated level of homocysteine (Hcy) [17.8µmol/l], cysteine, ethanolamine and ammonia in blood and only of Hcy in urine had been detected. High concentration of Methylmalonic acid (MMA) in urine[1631.4 mmol/mol creatinine] was detected by NMR spectroscopy of urine. In addition, CT scanning showed spina bifida aperta L5-S5.

The serum concentration of Hcy is elevated in both folate and cobalamin deficiencies, whereas MMA in serum or urine is a specific marker of cobalamin function. No mutation in MTHFR 677C-T in this patient was detected. The therapy with vitamin B_{12}[1mg/day], folic acid [5mg/day] along the restricted protein diet reduced the level of MMA in urine [to180.0 mmol/mol creatinine], of Hcy in blood and urine and improved the development of the child.
Further actions: Tests for mutations in MTHFR/1298A, MTRR, MTR, MMAA genes will be performed for this family. The NMR spectroscopy for both urine and amniotic fluid of the mother may be proposed for prenatal diagnosis. NMR spectroscopy of body fluids provided more analytic information in this case and may be used as screening method for diagnosis of inborn metabolic errors.

P14 Therapy for genetic disorders

P14.01
Discussions over a case of congenital diaphragmatic hernia

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Background: Congenital diaphragmatic hernia (CDH) is a birth defect associated with high mortality and morbidity during neonatal period. It has become increasingly apparent that genetic factors like retinoid-related genes may be involved in the pathogenesis of the disease. The need to increase the survival has pushed the limits of therapy to fetal surgery and endoscopic interventions. In severe cases, it is preferred nowadays to perform a temporary fetal endoscopic tracheal occlusion in order to stimulate lung development. Case report: We present a case of a male preterm child with right CDH. The CDH was diagnosed at 19 weeks of gestation and at 28 weeks a fetal endoscopic tracheal occlusion was performed. He was born by C-section at 32 weeks, birth weight of 3000 g. Even from birth the child suffered from severe hypoxia and pulmonary hypertension necessitating tracheal intubation and mechanical ventilation. During the first 24 hours of life the SaO2 remains under 80% despite the aggressive resuscitation, inotropic support, 100% O2, surfactant and Ilomedin administration. The patient died 36 hours after delivery because of severe pulmonary hypoplasia and pulmonary hypertension. Conclusion: Despite the modern and sometimes heroically treatment methods for CDH the final negative outcome is sometimes inevitable. Accurate medical lung development assessment is mandatory, before any attempt of surgical therapy. Sometimes pregnancy interruption should be considered as the best way to avoid sufferance.

P14.02
Combined strategies using airway clearence games and physical exercise in young pacients with cystic fibrosis

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Physiotherapy in cystic fibrosis techniques are often frustrating and exhausting for the child.
This study is aiming to demonstrate the need of respiratory clearance games and the importance of adapting these techniques to the CF children in order to increase compliance to therapy and quality of life.
Methods
We conducted a 12 months prospective study on 20 patients with CF aged between 4 and 12 years, from Romanian National Cystic Fibrosis Centre. They followed the protocol consisting in airway clearance games (flow-ball, TrainAir) and aerobic activities five times a week for 40 minutes. Children have been tested at inclusion and at the end of the study using the Questionnaire of life quality in CF. For data analysis we used Spearman's rho.
Results
After one year we observed an increase in sleep quality in 20% of cases and 30% of them enhanced the nutritional status. The majority of patients increased the level of fitness during ADL. Moderate fatigue in effort decreased from 60% to 50 % .Initially 40% of them followed constant educational activities compared to 60 % finally. Hospitalizations due to exacerbations decreased from 60% to 45% compared to the last year. Good correlation (at the 0.01 level) was observed between mucus quantity and fatigue during ADL, educational activities and sleep quality.
Conclusions:
Combined airway clearance games and physical exercises are a very good choice to optimize the quality of life in children with cystic fibrosis.
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P14.03
A pharmacogenetic analysis of Fabry patient responses to pharmacological chaperone treatment with AT1001 (GR181413A, Migalastat Hydrochloride)

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Fabry disease is an X-linked lysosomal storage disorder caused by mutations in *GLA*, the gene that encodes α-galactosidase A (α-Gal A). The pharmacological chaperone AT1001 (GR181413A, migalastat hydrochloride, 1-deoxygalactonojirimycin) is currently in Phase 3 clinical development as a potential therapy for Fabry disease. AT1001 selectively binds and stabilizes α-Gal A, increasing total cellular levels and activity for some mutant forms (defined as "responsive"). Responsive mutant forms can be determined readily in male Fabry patients; however, this is more challenging in female patients due to the expression of wild-type and mutant forms of α-Gal A. We assessed whether pharmacogenetic information could aid in the identification of Fabry patients with responsive mutant forms of α-Gal A. Fabry disease-associated mutant forms (473 in total) were individually expressed in HEK-293 cells; ~60% responded to AT1001 incubation. *GLA* mutations from the subset of mutant forms that responded to a clinically-relevant concentration of AT1001 (10 μM) were categorized as 'eligible'; others were considered 'ineligible'. Phase 2 clinical trial results were then retrospectively analyzed by grouping subjects, based on genotype, into these two categories. Oral administration of AT1001 increased α-Gal A more frequently in males with 'eligible' (13/14) versus 'ineligible' (0/3) mutations. Importantly, AT1001 treatment decreased kidney interstitial capillary globotriaosylceramide by ≥50% more frequently in male and female subjects with 'eligible' (7/10) versus 'ineligible' (2/7) mutations. Thus, the α-Gal A response measured *in vitro* for each mutant form may identify Fabry patients who can respond to AT1001 and are more likely to benefit from AT1001 therapy.

P14.04
In vivo quantification of cerebral $GABA_A$ receptors in fragile X patients using [^{11}C] flumazenil Positron Emission Tomography

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Fragile X syndrome is the most common form of inherited mental retardation. In the brain of fragile X animal models, we found a decreased mRNA expression of several subunits of the $GABA_A$ receptor. $GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the mammalian brain. This ionotropic receptor plays a role in learning and memory, hyperactivity, insomnia, epilepsy, anxiety and depression; processes that are disturbed in fragile X patients. This indicates that a dysfunction of the $GABA_A$ receptor is involved in the behavioural problems observed in the fragile X syndrome. The $GABA_A$

receptor is therefore a target for rational treatment of the syndrome. In a set of experiments we already demonstrated that GABAergic drugs are able to correct some of the symptoms in fragile X mice. However, before therapy on patients can be initiated, it is required to validate GABAergic abnormalities in human. As post-mortem studies are not feasible, we set up a study to image and quantify the $GABA_A$ receptor distribution *in vivo*. Using positron emission tomography (PET) with [^{11}C]Flumazenil, a known $GABA_A$ receptor antagonist, as a radioligand we determined the amount of $GABA_A$ receptors in the brain. We found an overall significant decrease (6-17%) in $GABA_A$ receptor availability in fragile X patients. The decrease was most prominent in specific brain regions, including cortical regions. This confirms that the GABAergic system is involved in the pathophysiology of the fragile X syndrome and suggests that GABAergic drugs might ameliorate several symptoms of fragile X patients, improving their quality of life.

P14.05
Creb1 is required for hydroxyurea-mediated induction of gamma-globin expression in K562 cells

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Hydroxyurea (HU) can augment gamma-globin expression in K562 cells. However, the transcription factors that are required for this upregulation have not been identified. Similarities between the HU and sodium butyrate (SB) pathways suggest Creb1 as a potential candidate. Reports indicate that in K562 cells, both HU and SB (1) activate p38 MAPK and (2) selectively induce Ggamma gene expression. In the case of SB, this induction seems to be mediated through a Creb1 binding site present in the promoter region of the Ggamma (and not Agamma) gene. In this study, we have investigated the possible role of Creb1 in the HU pathway. We show that transient or stable knockdown of Creb1 blocks HU-mediated gamma-globin induction in K562 cells. However quantitative ARMS-PCR (Amplification Refractory Mutation System) experiments suggest that HU does not solely induce Ggamma expression in this cell line. Electrophoretic mobility shift assays (EMSAs) indicate that HU treatment leads to increased levels of Creb1 complexes binding to the Ggamma promoter. Collectively, these results suggest that Creb1 is necessary for gamma-globin induction by HU, a role which may in part be mediated through the Ggamma promoter Creb1 binding site.

P14.06
Hemangioma of infancy: treatment options

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Background: Hemangiomas of infancy (HOI) are the most common tumors in children. Even that HOI are not primarily genetic diseases, there are several genes and loci implicated in the pathogenesis of vascular malformations and hemangiomas whit major influence over the clinical characteristics and evolution of hemangiomas: RASA1, KRIT, TIE2/TEK, ENG, SMAD4/MADH4. Material and method: This study is an analysis of all the cases with the diagnostic of HOI admitted in our hospital from January 2008 to December 2010 Results: There were a total number of 134 patients with age ranging from 2 months to 8 years. In 54.3 % of the cases were localized on the neck and head, 22.7% on the torso, 13.86% on the limbs, 3, 24% on the genitals, and multiple in 5.9%. Surgical excision was the most common procedure followed by Aethoxysclerol injection. In 8 cases we used a new therapy: propranolol. The outcomes of treatment were good in the majority of the cases with no major complications, except one case of keloid formation after the excision of a very large hemangioma. Conclusions: The treatment of hemangioma varies according to affected area. In most of the cases surgical excision is sufficient. For large hemangioma and those located in esthetic areas the most promising treatment is now propranolol. Aethoxisclerol is used only when propranolol therapy has no results and the surgical excizion is prohibited. Feature research should be focused to correlate the treatment with the genetic factors influencing the clinical course of HOI.

P14.07
One year-treatment with a combination of pravastatin and zoledronate on six patients affected with Hutchinson-Gilford Progeria: safety and efficacy.

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Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare and severe genetic disease, characterized by segmental premature aging and accelerated atherosclerosis. It invariably leads to death in the teenage, due in most cases to myocardial infarction. No validated treatment is available to date. A recurrent, *de novo,* dominant mutation of the *LMNA* gene, encoding Lamins A/C, causes most HGPS cases, through the activation of a cryptic pre-mRNA splice site leading to the production of a truncated Lamin A precursor called "progerin". Progerin cannot be fully post-translationally processed, remains aberrantly prenylated and accumulates in cells' nuclei, where it exerts several toxic effects. Preclinical studies *in vitro* on patients' cells and on animal models provided proof-of-principle that the combined use of pravastatin, a statin, and zoledronate, an aminobisphosphonate, could improve several disease parameters, including growth, bone density and survival. These data allowed us to launch a phase II, open-label, single arm clinical trial, conducted in Marseille, France, on 12 European children affected with Progeria. The trial aims to assess the safety and efficacy of a combination of pravastatin and zoledronate on several pathological parameters, including growth, bone density and turnover as well as cardiovascular risk. Data obtained in six children treated for one year showed that the association of pravastatin and zoledronate is safe and can induce clinical beneficial effects in the mid-term. We thus report the first therapeutic intervention that seems to favorably impact the natural course of this devastating disease, with potential wider impacts in the field of aging.

P14.08
Association of Estrogen receptor α -351 XbaI A>G and -397 PvuII T>C Polymorphisms with uterine leiomyoma in women from Chaharmahal va Bakhtiari province; Iran

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Background and Aims: uterine leiomyoma, or fibroid, is common estrogen-related gynaecological disease, and it is estimated that one in four women during reproductive period will develop this kind of benign neoplasia. We aimed to elucidate the association of estrogen receptor a (ERα)-351 A>G (XbaI) and -397 T>C (PvuII) gene polymorphisms with leiomyoma in a group of women from province in southwest of iran.

Methods: 156 women with clinically diagnosed uterine leiomyoma and 151 healthy,normal women were included in the study. Genomic DNA was obtained from peripheral leukocytes. ERα-351 A/G XbaI and -397 T/C PvuII polymorphisms were assayed by the method of PCR-RFLP.

Results: Genotypes and allelic frequencies in each group were compared. The genotype/allele frequencies of Era -351 A>G and -397 T/C polymorphisms in leiomyoma groups were not different from those of normal controls.

Conclusion: We concluded that ERα -351 XbaI A>G and -397 PvuII T>C related genotypes/alleles were not correlated with an increased risk of uterine leiomyoma in study population.

P14.09
Towards lentiviral gene therapy for the treatment of Pompe disease

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The lysosomal storage disorder Pompe disease is due to a deficiency of the acid alpha-glucosidase (GAA) gene, and characterized by progressive muscle weakness, in its most severe form lethal in the first year of life due to cardiac and respiratory failure. Enzyme replacement therapy is effective, but not beneficial to all patients and often results in antibody formation. We have used a hematopoietic stem cell *ex vivo* lentiviral gene therapy approach in the Pompe mouse model using the native or a codon-optimized GAA transgene driven by the spleen focus forming virus promoter.
Using this strategy, sustained levels of GAA were achieved in leukocytes and other affected tissues of Pompe mice. Using the native sequence, glycogen storage was reduced significantly in heart and diaphragm, with near normalization of heart morphology and function, but only to a limited extent in skeletal muscle. Challenge of the treated animals with the rhGAA protein demonstrated robust specific immune tolerance. The use of the codon-optimized GAA gene (GAAco) resulted in even higher levels of GAA activity in all affected tissues, including skeletal muscle and an almost complete reduction of glycogen storage. Using the GAAco construct, skeletal muscle function was maintained and initial results also indicate reduced glycogen levels in brain tissue.
Thus, *ex vivo* hematopoietic stem cell lentiviral vector mediated gene therapy results in correction of the Pompe phenotype in the mouse model and would be suitable for further clinical exploration.

P14.10
Increased T allele frequency in multidrug transporter ABCB1 (MDR1) gene in FMF patients of colchicine unresponsiveness

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The multidrug resistance gene-1 (MDR1, ATP-binding cassette transporter: ABCB1, P-glycoprotein: P-gp) encodes membrane proteins that playing a crucial role in protecting cells from xenobiotics, chemicals and drugs. The TT genotype of 3435 codon in exon 26 of MDR1 gene causes to over-expression gene activity and efflux many chemically diverse compounds across the plasma membrane. We studied the association between C3435T polymorphisms (SNP) of MDR1 gene and colchicine-resistant FMF patients. Total genomic DNA samples from 52 FMF patients of colchicine unresponsiveness were used for Familial Mediterranean Fever (MEFV) and MDR1 genes profile analyses. Target genes were genotyped by multiplex PCR based reverse hybridization stripAssay method. The preliminary current results showed that increased T allele frequency (60%) in FMF patients of colchicine unresponsiveness. The distributions of the CC, CT and TT genotypes in colchicine nonresponder FMF patients were 17%, 46%, and 37%, respectively. Our results indicate that C3435T polymorphism in exon 26 of MDR1 gene is associated with colchicine resistance in nonresponder FMF patients during the common therapy protocol.

P14.11
Development of the exon exchange method for repair of mutant nebulin transcripts

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The nebulin gene (*NEB*) has 183 exons encoding transcripts up to 26 kb in length. Mutations found in *NEB* are dispersed throughout the gene, i.e. no mutational hot spots are evident. Mutations cause autosomal recessive nemaline myopathy, distal nebulin myopathy and core-rod myopathy, for which no therapy is available. The size of *NEB* limits the options of gene therapy development. Thus, our research has focused on methods correcting the mutation carrying transcripts. Recent advances in RNA reprogramming technologies have raised the possibility to develop RNA therapies for correction of mutations in *NEB* at the pre-mRNA level. Spliceosome-mediated RNA *trans*-splicing (exon exchange), i.e. splicing between two separate pre-mRNA molecules, allows the replacement of mutant exons with wild type ones at the pre-mRNA level. Targeted exon exchange requires the spliceosome and a target pre-mRNA, both provided by the cell, and a pre-*trans*-splicing RNA-molecule (PTM) which is produced from an expression vector transfected into the cells. PTMs can be designed to carry out three forms of exon exchange: 3'-, 5'- or internal exon replacement. We have developed PTMs for *NEB* 3'- and internal exon replacement. PTMs designed carry one or several wild type *NEB* exons to replace exons from target pre-mRNAs produced from *NEB* minigene expression vectors in cell cultures. We have obtained successful results from our 3'exon exchange and first internal exon exchange experiments. We are now focusing on further development of the internal exon replacement method, which would be the most useful one for correction of mutations in *NEB* transcripts.

P14.12
Stem cell therapy of a genetic model of neurodegeneration with cerebellar ataxia: the Harlequin mouse

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The treatment of neurodegenerative disorders represents one of the critical goals of medical research today. Although progressive neurodegenerative diseases are commonly associated with ageing, they also affect children and young adults. Cerebellar ataxias are rare neurodegenerative disorders, which can be thought of as a syndrome, which has many different causes, genetic or environmental. These diseases are progressive and very serious illnesses with no treatment available so far. A common feature in the early stages of these diseases lies in mitochondrial dysfunction and oxidative stress induced by a deregulation of oxidative phosphorylation.
The Harlequin (Hq) mouse, where a spontaneous mutation in the AIF gene results in a drastic reduction of its expression, gradually develops a cerebellar ataxia associated with mitochondrial degeneration and a complex I deficiency. Although all cerebellar neurons finally die after 6 months, granule neurons are affected first with a gradual death starting around 2 months.
We have designed an in vitro protocol combining media and selective factors to push hES toward the neuronal lineage and make them differentiate into functional neurons expressing granular markers of the cerebellum. We have also produced granule neuron progenitors to attempt cell replacement in ataxic Hq mice. We show that progenitors implanted in the cerebellum can differentiate into mature human neurons and integrate into the adult mouse cerebellum. Motor coordination was improved in cell-treated animals up to three months after implantation. Our results bring a proof-of-principle that human embryonic stem cell-based therapies might be relevant for treating neurodegenerative disorders.

P14.13
Oxidative stress and sperm DNA quality in couples experiencing recurrent spontaneous abortions

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Introduction: Sperm has dynamic and critical role in embryogenesis that extends clearly beyond fertilization. Supraphysiological Reactive Oxygen Species (ROS) levels damage both mitochondrial and nuclear genome. During embryonic development, nucleotide alterations if unrepaired are lethal to accurate transmission of genetic information and affect the embryonic viability and post natal health. Nicks/breaks and/or nucleotide modification caused by unregulated free radical concentration due to inefficient antioxidant machinery leads to sperm DNA damage. This study was planned to understand the association of seminal ROS, antioxidant capacity and sperm DNA damage in men of couples experiencing recurrent pregnancy loss following spontaneous conception.

Material and Methods: Male partners of 77 couples experiencing recurrent pregnancy loss and 41 fertile controls were included in study. Seminal ROS were measured by luminol induced chemiluminescence. Seminal total antioxidant capacity (TAC) was assessed by commercially available kit. Sperm DNA integrity was quantified by single cell gel electrophoresis (comet assay). Semen analysis was done according to WHO 2010 guidelines. Mann-Whittney test was applied for statistical significance.
Results: Significantly increased ROS and reduced seminal TAC was observed in patients (p=0.002; p=0.014 respectively) as compared to controls. In patient group the number of sperms with DNA damage was higher (p=0.024).
Discussion: Seminal oxidative stress is a major cause of sperm DNA damage. DNA damage leads to recurrent spontaneous abortions, childhood cancers, genetic and epigenetic defects in offspring. Oxidative stress and sperm DNA damage assessment have better diagnostic and prognostic capabilities and should be included in diagnostic workup of patients experiencing recurrent pregnancy loss.

P14.14
Strategies for rehabilitation in a case with Russel-Silver syndrome

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Russel-Silver syndrome is a genetically heterogeneous disorder, characterized by prenatal and postnatal growth retardation, characteristic facial appearance, slow regressive evolution, risk of delayed development and learning disabilities. We present the case of a 1.7 years old girl, born from healthy, unrelated parents, hospitalized for treatment and functional rehabilitation. The birth was premature, by caesarean section at 36 weeks, after a pregnancy with early and late dysgravidy, birth weight 1900g. Clinical and functional evaluation at admission revealed hypotrophy, moderate motor retardation, characteristic facies, delayed developmental milestones, axial hypotonia, dorso-lumbar kyphosis accentuated in sitting position, symmetric muscle hypotrophy, balance disturbance, standing and sitting only with help, walking only sustained with a broad base of support, stability problems, mental development within ranges of chronological age. Rehabilitation treatment was complex and involved coordination of several team members. The main goals of the treatment were increasing the muscle tonus of the limbs, obtaining independent standing, establishment of stability for ambulation, walking, motor skills acquisition, posture amelioration, increasing tonus of paravertebral, abdominal muscles, body alignment. Intensive rehabilitation program consisted of hydrotherapy and aquatic therapy in thermal water, electrotherapy, general tonifiant daily massage, physical, occupational and speech therapy. She showed significant improvement, after three weeks of intensive treatment walked a few steps independently, but falled frequently, improved appetite, gained weight, improved muscle tone, climbed stairs independently, with support. Treatment normally involves massive amounts of stimulation in order to improve muscle function and control. Assessment of these cases is best accomplished by a multidisciplinary team.

P14.15***
Cysteine quantity correction in CADASIL using antisense mediated exon-skipping.

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most prevalent type of hereditary vascular dementia and is recognized as an important cause of stroke and dementia in the young. CADASIL is caused by characteristic missense mutations of the *NOTCH3* gene which lead to an uneven number of cysteine residues in the protein's ectodomain. This causes misfolding of the protein which leads to aggregation of NOTCH3 at the surface of vascular smooth muscle cells, a key factor in CADASIL pathophysiology
We are currently testing a novel application for antisense-mediated protein modulation, named 'cysteine quantity correction', as a treatment strategy for CADASIL. In this approach we use antisense mediated exon-skipping to intervene in protein synthesis at the pre-mRNA level. By inducing the skipping of specific exons, we re-establish an even number of cysteine residues in the NOTCH3 protein's ectodomain. Our hypothesis is that by this modulation the protein maintains function, but loses toxicity. With this approach, we can target ca. 70% of CADASIL related mutations using the same set of antisense oligonucleotides.
Technically, we are able to induce the desired exon-skipping in our CADASIL cell models as shown by RT-PCR and sequencing analysis. Currently, we are evaluating whether the induced cysteine quantity correction reduces CADASIL associated NOTCH3 accumulation *in vitro*, using Western blot and immunocytofluorescence.
Our hypothesis and preliminary, but promising, *in vitro* results will be presented.

P14.16
Production of K562 cells stably expressing MBD2 shRNAs

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MBD2 (Methyl Binding Domain 2) has been shown to repress the human γ-globin gene in mouse models. We have previously shown that transient MBD2 knockdown (35%) using siRNAs results in induction of γ-globin expression (1.75 fold) in K562 cell line. In order to achieve higher levels of knockdown and possibly g-globin induction, a lentivirus system was used for shRNA delivery. First, functionality of the system was verified by using a lentiviral transfer vector containing a GFP expression-cassette. Next, five lentivirus transfer vectors containing MBD2 shRNAs were screened for target gene knockdown by qRT-PCR in HEK 293T transient transfection assays. The two most effective shRNAs gave >70% mRNA knockdown levels after normalization for transfection efficiency. These vectors were subsequently packaged and used to transduce K562 cells. Upon infection and antibiotic selection, a cell population stably expressing MBD2 shRNAs was obtained. The amount of MBD2 knockdown and γ-globin expression was assessed in these cells by relative qRT-PCR. In one cell line (shRNA-3), MBD2 was knocked down by 73% and g-globin was induced 5-fold. In the other (shRNA-5), MBD2 was down-regulated by 51% and g-globin increased by 11.75-fold. These results support those of the transient RNAi experiments. Moreover, these cell lines can now be used for delineating the mechanism of MBD2 repression.

P14.17
Enzyme replaced therapy in MPS II patients in Novosibirsk.

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Intention: To demonstrate clinical changes and objective and subjective improvement in health status in patients with mucopolysaccharidosis II (MPS II) after enzyme replacement therapy (ERT).
Materials and methods: Four patients with MPS type II (16, 13, 6, and 5 years old) who had been treated with Elaprase intravenous infusion weekly over a 54-week period. The urinary GAG level decreased to the initial value. A multidisciplinary physicians checkup including 6-MWT, ECG, computed tomographic scanning, US of the abdominal cavity, echocardiography, respiratory function test, clinical blood and blood serum biochemistry was performed before and after the therapy.
Results: Two children aged 5-6 years showed a general improvement of movement abnormalities and lung function in contrast with adult patients. A progressive arthropathy temporarily remitted in the 16 year old patient; there was no effect in the 13 year old. Mitral and aortic regurgitation and/or stenosis commonly decreased after ERT. US of the abdominal cavity did not show significant decreased hepatosplenomegaly in the patient aged 13.
Idursulfase is generally well tolerated, although infusion reactions did occur.
Conclusion: The response to ERT appears to depend on the severity of the
individual's condition and the age at which the treatment begins. Infusion reactions were observed in the older patients despite allergic problems which all children had before Elaprase treatment. Our experience demonstrates the first sign of ERT efficacy in most young

patients and increases their ability to take part in normal daily activities.

P14.18
Expression of pdx1 promote derivation of beta cells from mesenchymal stem cells

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The Pancreatic cells transplantation is a promising approach for treatment of type1 diabetes that caused by insulin deficiency; however, lack of suitable donors limits the application. Differentiated mesenchymal stem cells (MSCs) can be therapeutic source for the cure of type 1 diabetes. The aim of current experiment is to explore the possibility of derivation of insulin producing cells from bone marrow MSCs by overexpression of pdx1 transcription factor. In this study rat MSCs were isolated and identified by flow cytometry. Then, MSCs were transduced by lentiviruses harboring pdx1 and pdx1-expressing cells selected by puromycin. The appropriate expression of exogenous pdx1 was confirmed in level of mRNA and protein using RT-PCR and immunofluorescent analyses. In addition to ectopic expression of pdx1, differentiating medium containing nicotinamid and betamercaptoethanol (Nico/betaME) used to efficient differentiation of MSCs into beta cells. Then, the expression of islet markers was investigated by quantitative RT-PCR. The immunoflurescent showed a nuclear localization of pdx1. Pdx1-expressing MSCs transcribed specific pancreatic endocrine markers such as endogenous Pdx1, Ngn3, Glucagon and insulin regardless to using Nico/betaME, but the quantitative RT-PCR showed a high increase in all markers except insulin, in Nico/betaME treated cells compared to non-treated cells. The beta cells derived from MSCs also expressed glucokinase and Glut2 indicating these cells have the glucose sensing ability. In contrast, treated MSCs didn't express P48; it implies these cells have been committed to endocrine linage. In conclusion, MSCs as an available resource can have impacted in cell based gene therapy of type1 diabetes.

P14.19
PTC124 incubation towards ribosomal readthrough of nonsense mediated termination codons in the SLC6A8 and GAMT genes.

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A recently discovered chemical entity, PTC124 (Ataluren) was shown to selectively induce ribosomal readthrough of premature but not normal termination codons. In vitro experiments and subsequent treatment of mdx mice showed a promising improvement in the form of functional recovery (Welch et al. 2007). This led us to investigate the effect of PTC124 on nonsense mediated premature termination in two of the three cerebral creatine deficiency syndrome genes, namely the creatine transporter gene (SLC6A8) and the guanidinoacetate methyl-transferase gene (GAMT). We obtained PTC124 from Selleck Chemicals (Houston, Texas, USA) and incubated patient's cell-lines containing nonsense mutations with 17μM of PTC124 for 46 days (SLC6A8, n=2) and 14 days (GAMT, n=3) and collected cells every 7 days. We measured intra-cellular creatine content and formation of 2H3-13C2-creatine using a two-step derivatization procedure, followed by quantification with GC-MS. With all cell-lines we found no increase of function, indicated by low intra-cellular creatine content (SLC6A8) (Rosenberg et al. 2007) and no formed 2H3-13C2-creatine (GAMT) (Verhoeven et al. 2004) as compared to the wild-type cell-lines. This led us to conclude that PTC124 (Selleck Chemicals) cannot induce ribosomal readthrough of the five tested nonsense mediated termination codons in the SLC6A8 and GAMT gene. However, since we were unable to validate the obtained compound, these results could also have been caused by the initial inability of our PTC124 to function correctly.

P14.20
Inhibition of superoxide dismutase expression with progestins as a mechanism for the treatment of familial amyotrophic lateral sclerosis.

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Familial amyotrophic lateral sclerosis (fALS) is a severe motoneuron disease, and mutations in superoxide dismutase (SOD1) are responsible for at least 20% of cases. fALS is inherited in an autosomal dominant manner and disease severity is mutant gene dose-dependent. Knockout of the SOD1 gene does not result in motoneuron disease in the mouse. Thus reduction of mutant SOD1 expression has been proposed as a mechanism for treatment of fALS. We aimed to identify compounds that selectively decrease cellular levels of SOD1. A library of known drug-like molecules was screened for the ability to decrease SOD1 levels in several cell lines including Hek-293 cells and HeLa cells. Cells were seeded into 96-well plates and test compounds were added 24 hours later. Several progestins (IC50: 100-300 nM) dose-dependently decreased SOD1 levels following 96 hours of incubation. Total cell number, cell morphology, and total protein level was not affected in the transformed cell lines. However, in HeLa cells, all progestins exhibited significant cytotoxicity, as expected based on the reported toxic effect of siRNA to SOD1 in specifically cancer-derived cells. Norethindrone, a progesterone analog, selectively decreased SOD1 cellular levels by 80% (IC50: 92 nM). The progesterone receptor antagonist mifepristone dose-dependently inhibited this effect, supporting a receptor mediated action. In the G93A mouse model of fALS, 7 days intraperitoneal administration of norethindrone (100 μg/Kg) decreased spinal cord levels of mutant human SOD1 in preliminary studies. Collectively, these results suggest that further investigation of progestins in the treatment of fALS is warranted.

P14.21
Differential response to VPA in SMA therapy: Did we catch the bad guy?

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Proximal spinal muscular atrophy (SMA) is the number one genetic killer during early infancy. It is caused by functional loss of SMN1, resulting in progressive degeneration of spinal α-motor neurons. SMA severity is mainly determined by SMN2 copy number. Consequently, a number of therapeutic approaches has been developed directly targeting SMN2. We have shown that valproic acid (VPA), a short-chain fatty acid histone deacetylase inhibitor (HDACi), significantly increases SMN levels in vitro, ex vivo and in VPA-treated SMA patients. However, in a first pilot clinical trial with VPA only 1/3 of the patients exhibited markedly increased SMN levels, whereas the remaining were non-responders. To identify the crucial factors accounting for this observation, we established fibroblast lines from >30 SMA-patients undergoing VPA-therapy. Response to VPA was concordant between blood and fibroblasts in about 65% of cases. By microarray analysis we identified four significantly differentially expressed transcripts between both patient-groups. Strikingly, non-responders lacked any differences in expression between mock- and VPA-treated cells, suggesting a failure of HDAC inhibition. Indeed, the most differentially expressed gene is involved in fatty acid transport suggesting an altered VPA import/export. By employing different analytical and biochemical methods, we could identify striking differences in fatty acid uptake and metabolism, which most likely account for the observed differential response to VPA.

Our data sheds first light on the molecular mechanisms influencing VPA response and may not only have a major impact on the therapy of SMA but also on other diseases treated with VPA, like epilepsy or migraine.

P14.22
Genotoxicity biomarkers in thalassaemic patients

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Introduction: Iron plays a role in carcinogenesis through the generation of oxygen free radicals. In thalassemic patients that do not receive transfusions, abnormal iron absorption produces an increase in the body iron. Regular blood transfusions lead to a greater iron accumulation, therefore it is essential an iron chelation therapy.
Purpose: To evaluate the efficiency of iron chelating therapy to prevent genotoxicity in polytransfused thalassemic patients, we analysed lymphocytic DNA damage by Micronucleus test (MNT) and Comet assay.
Methods: The study includes 77 transfusions-dependent β thalassemic patients and a control group of 20 no transfused subjects with thalassemia intermedia. The patients were divided in four therapy groups: 25 were treated with Deferasirox (ICL670), 23 with Deferoxamine (DFO), 10 with Deferiprone (L1) and 19 with combined therapy (DFO+L1).
Results: The results are summarized in Table 1. Micronuclei distribution is different in four groups (Kruskal-Wallis test, p=0.014). No significant differences were observed using the Comet assay for the high intra-group variability.
All groups showed a significant difference compared with the control group (Mann-Whitney U test, p<0.05) for MNT, except for the patients with ICL670 therapy.
Discussion: In the examined sample, ICL670 is the most effective, followed by the DFO and combined therapy.
The Deferiprone would seem the least effective but needs a more numerous patients group.

Table 1: The results for Micronucleus test and Comet assay

	NO Therapy	ICL670	DFO	L1	DFO+L1
MN	13.3 ± 6.4	14.5 ± 7.8	17 ± 6.2	24.1 ± 9.4	18.4 ± 15.2
Tail DNA %	11.4 ± 8.5	14 ± 12.7	11.9 ± 7.9	14.4 ± 12.3	14.2 ± 13.2

J14.01
Stem cell a new way in treatment hearing loss impairment.

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Deafness is a condition that has an enormous personal, social and economical impact.
The options available to treat are very limited, and represented by prosthetic devices such as hearing aids and cochlear implants. There is a clear need for a therapeutic breakthrough that will help the millions of people affected, so studies in stem cell technologies have been initiated in order to repair, regenerate or replace lost hair cells
The auditory organs appear to contain cell with stem cell properties that can differentiate to hair cells and neurons.
Embryonic stem cells are one potential source. Neural progenitors made from embryonic stem cells, transplanted into the inner ear, they differentiated in to cells that expressed neuronal markers. The re growth of these neurons suggests that it may be possible to replace audiotory neurons that have degenerated with neurons that restore auditory function.
Stem cell therapy to treat deafness is still some years in to the future because of; first, different cell types are potentially to be used, all of them having advantages and disadvantages. Second, in order to target such a small and secluded organ as the cochlea, difficult surgical techniques are to be used, some of which still need to be developed.
But we sure combination of stem cell, gene therapy and drug treatment in conjunction with technical devices, such as microprocessor-controlled cochlear-interface implants can start a new avenue for the development of strategies to restore hearing therefore they are offering a glimmer of hope for these afflictions.

P15 Laboratory and quality management

P15.01
Bacterial DNA interference with aged bone DNA amplification

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Introduction: Molecular human identification of remnants of human body is of increasing usage in natural disasters, wars and forensic laboratories. Bone and teeth are major source for genomic DNA due to their higher stability. DNA degradation and also contamination with PCR inhibitors could interfere with DNA amplification and contamination with bacterial DNA might be a reason especially when we work with aged DNA (aDNA). Here we report effect of bacterial DNA contamination in DNA extracted from Iranian human remains from the war with amplification using real-time PCR.
Material and methods: DNA was extracted from aged bone (remained from 20-30 years before) and also E.coli. PCR based techniques were used to amplify both human and bacterial DNAs separately using different set of primers. aDNA was mixed with bacterial DNA and amplification was performed using both set of primers.
Result: Amplification of artificially contaminated aged bone DNA with E. coli DNA didn't showed any negative interference or inhibition by bacterial DNA. Even in some samples, amplification was performed just in the cases of mixture aDNA with bacterial DNA. We tested this with real-time PCR and conventional PCR results were confirmed by amplification of mixed DNA in 2-3 cycles before the aDNA.
Conclusion: It seems bacterial DNA does not inhibit amplification of human aDNA. Even in some instance it might act as a biological indicator for the presence or absence of PCR inhibitor.

P15.02
Validation of two commercially available kits for clinical Rapid Aneuploidy Detection

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Rapid aneuploidy detection (RAD), allows the rapid and efficient detection of the common aneuploidies in at-risk pregnancies. RAD, although widely used in Europe, is only beginning to emerge as a front-line prenatal test in North America. Our experience with 161 samples demonstrated that Aneufast™ v1 (Molgentix SL) required a high rate of reflex testing (10.2% of the chromosome pairs tested were non-informative or out-of-range representing 29% of all patient samples). In an effort to decrease cost and sample reporting time, we chose to validate Aneufast® v2, released in January 2011 and Elucigene's Q*STR*plus*V2 (Gen-Probe). A series of 48 samples were chosen: 24 with confirmed aneuploidies (karyotype and Aneufast™ v1), 2 normal controls, and 24 normal samples that required reflex with Aneufast™ v1. All samples were analyzed according to manufacturer's recommendations with 2 informative markers required for classification. Our results demonstrated that the amount of reflex testing in the validation dataset is dramatically reduced: 5.7% of chromosome pairs (20.8% of patient samples) for Aneufast® v.2 while Q*STR*plus*V2 required reflexing in 3.1% of chromosome pairs (12.5% of patient samples). The sensitivity and specificity of all three tests was identical (sensitivity = 95.5%, specificity = 100%). Importantly,

the Q*STR*plus*V2 kit, using the TAF9L microsatellite, determined the number of copies of the X chromosome present in the initial test while Aneufast® v2 required reflex to obtain the TAF9L ratio. Therefore, in our experience, the Q*STR*plus*V2 kit has a lower overall time to report and is more cost-effective.

P15.03
Twenty years of Molecular Genetics External Quality Assessment

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UK National External Quality Assessment Scheme (UKNEQAS) for Molecular Genetics has been providing a measure of the standard of molecular laboratory services for the last twenty years. The scheme started in 1991 with a few laboratories participating in genotyping only external quality assessments (EQAs) for CF, HD and DMD. This has developed into a self-funding, not-for-profit organisation which now offers 29 separate EQAs to over 120 participating laboratories.

EQAs assess genotyping accuracy, result interpretation and clerical accuracy of the reports. Samples are distributed and participants are required to submit interpretative reports in the laboratory's standard format. This ensures that the whole process is assessed, from sample receipt to the issuing of the final report. Performance monitoring has identified errors in different steps of the process providing evidence that this assessment of the process is essential in measuring the standard of the laboratory. Web-based interpretative EQAs have been established for rarer diseases to reduce EQA costs for laboratories. Wherever professional Best Practice Guidelines are available these are used to establish the marking criteria for each EQA.

Six key areas are covered by these EQAs; core diseases, preimplantation genetic diagnosis, newborn screening, non-invasive prenatal diagnosis, molecular rapid aneuploidy testing (UKNEQAS for Cytogenetics collaboration) and molecular histopathology for *KRAS* and *EGFR* testing (UKNEQAS ICC collaboration).

Performance is high in well established EQAs but pilot EQAs identify a number of performance issues. As laboratories participate more regularly in EQAs then there is a marked improvement in performance and the standard of laboratory output.

P15.04
Measuring the improvement of the interpretation in cystic fibrosis laboratory reports

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Interpretation of genetic test results in laboratory reports is an ISO and OECD requirement. It should be performed according to the indication for testing and considering that the recipient may not understand all implications of genetic test results. We assessed interpretation aspects for similar cases circulated through the Cystic Fibrosis (CF) External Quality Assessment (EQA) scheme in 2004 (n=232 laboratories), 2008 (n=202) and 2009 (n=203). The mock case described a newborn with meconium ileus at birth; a molecular analysis was requested to confirm the CF diagnosis. The presence of two mutations indeed confirmed the diagnosis. "Confirmation of diagnosis" was present in 90% of the reports in 2004, 95% in 2008 and 97% in 2009. "Recommendation for genetic counselling" was included in 85%, 90% and 89% of reports respectively. "Suggestion for prenatal or preimplantation diagnosis for subsequent pregnancies" was mentioned in 54%, 67% and 75% of reports respectively. The average interpretation score was 1.49/2.00 in 2004, 1.77/2.00 in 2008 and 1.85/2.00 in 2009. The score for laboratories that participated in the three years (n=132) evolved from 1.52/2.00 to 1.80/2.00 and 1.90/2.00. Those that participated only once scored 1.47/2.00 in 2004 (n=58), 1.67/2.00 in 2008 (n=15) and 1.51/2.00 in 2009 (n=18). In 2004, 22% of the participants obtained 2.00/2.00, compared to 66% in 2009. This study demonstrated that interpretation improved over the years, in particular regarding implications for all genetic counselling aspects, and that regular participation to EQA contributes to an improved quality of reporting.

P15.05
The Italian External Quality Assessment in classical cytogenetics: state of the art

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The Italian External Quality Assessment (IEQA) in classical cytogenetics started in 2001 and is coordinated by the Istituto Superiore di Sanità, (ISS). The IEQA covers both prenatal and postnatal diagnosis, including cancer cytogenetics.

From 2001 to 2009 this activity has been funded by the Italian Ministry of Health through research projects and participation to the IEQA was free. Since 2010 laboratories pay a fee for each scheme and participation is not anymore restricted to public genetic services only (Gazzetta Ufficiale Serie Generale n.199-28th August 2009).

The IEQA is retrospective since laboratories submit images and reports of two cases they have already analysed; cases are selected according to a fixed schedule. In 2008 a web-based system was developed; all EQA phases are managed through three different sections dedicated to the Scheme Organizer, the participants and the assessors.

Assessment takes into account technical, analytical and interpretative performance and two different groups of assessors are involved in constitutional and cancer cytogenetics. A clear marking system has been established and agreed by the Scheme organiser and assessors in order to better identify the performance of laboratories. Following assessment each participant receives a summary letter and an individual report with scores and comments.

Results of the VIth (2008-2009) and VII th trial (2010) will be showed and discussed.

P15.06
European external quality assessment for the improvement of *KRAS* testing

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KRAS mutational status in tumor cells has become an important determinant for the successful application of epidermal growth factor receptor targeting therapy in patients with colorectal cancer. In an effort to ensure optimal, uniform and reliable community based *KRAS* testing throughout Europe, a *KRAS* External Quality Assessment (EQA) scheme was set up. In total 76 laboratories from 14 different European countries participated and were divided among 6 regional schemes. For each scheme, one regional scheme organizer prepared and distributed the samples, in accordance with agreements on the mutational status. The samples included unstained sections of 10 invasive colorectal carcinomas with known *KRAS* genotype. The samples were centrally validated by a reference laboratory. From the 76 participating laboratories, 50 reported all 10 genotypes correctly (66%). Thirteen laboratories genotyped one out of 10 samples wrong and 3 laboratories genotyped two or more out of the 10 samples wrong. Ten laboratories reported a technical error (no result) for one or more samples. The average genotyping score for the *KRAS* EQA scheme is 9,49/10,00 (94,9%). There is an overall improvement in the score of laboratories that participated also in the *KRAS* EQA scheme of last year. Mistakes were made both in laboratories using commercial and "in-house" validated assays. In addition, we analysed tumor percentage analysis and written reports for educational purposes.

This EQA scheme is a useful tool to provide information about the performance of a given laboratory compared to others, and to stimulate optimalisation of lab procedures.

P15.07
Evaluation of tripled repeat primed (TP)-PCR assay and Sizing PCR assay of the FMR1 gene for the molecular diagnosis of the fragile X syndrome

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Fragile X syndrome is caused by the abnormal expansion of the CGG repeat in the 5' untranslated region of the FMR1 (Fragile X Mental Retardation 1) gene. Routine molecular analysis includes a combination of PCR and Southern blot analysis. However, Southern blot analysis largely constrains throughput and efficiency and requires large amounts of high quality DNA.

This study aims to evaluate if in a routine diagnostic setting Southern blot can be replaced by PCR based assays. We evaluated the FMR1 TP (triplet repeat primed)-PCR assay (Abbott) for rapid distinction between normal and expanded FMR1 alleles and the FMR1 Sizing PCR assay (Abbott) for accurate sizing of alleles.

We evaluated assay performance using DNA from 4 cell lines and 53 archived samples (from 9 normal individuals, 1 case with a grey zone allele, 13 pre- and 31 full mutation carriers with repeat expansions ranging between 57-1300 CGG, 4 mosaic cases with alleles within the pre- and full mutation range). The TP-PCR assay accurately distinguished normal from expanded alleles. The Sizing PCR assay accurately sized premutation and full mutation alleles up to 335 CGG repeats. Interpretation of some mosaic cases was challenging with TP-PCR.

In conclusion, the assays evaluated allow efficient workflows in a diagnostic context and lead to a final conclusion for the large majority of the referred cases. A methylation-sensitive test is still required to unequivocally distinguish premutation from full mutation alleles with lengths in the transition zone (150-250 CGG), where correct interpretation depends on methylation status.

P15.08***
Discrepancies in reporting the CAG repeat lengths for Huntington's disease

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Huntington's disease results from a CAG repeat expansion in the first exon of the *Huntingtin* gene; this is measured routinely in diagnostic laboratories. The European Huntington's Disease Network REGISTRY project centrally measures CAG repeat lengths from fresh blood samples; these were compared with the original results from 121 laboratories across 15 countries. We report on 1326 duplicate results: there was a discrepancy of reporting the upper allele in 51% of cases; this reduced to 13.3% if acceptable measurement errors proposed by the American College of Medical Genetics were applied. Duplicate results were available for 1250 lower alleles and discrepancies occurred in 40%. Clinically significant discrepancies occurred in 4.0% of cases with a potential unexplained mis-diagnosis rate of 0.3%. There was considerable variation in the discrepancy rate among 10 of the countries participating in this study. 348/1326 DNA samples were re-analysed by an accredited diagnostic laboratory based in Germany, with concordance rates of 93% and 94% for the upper and lower alleles respectively. This became 100% if the acceptable measurement errors were applied. The central laboratory correctly reported allele sizes for 6 standard reference samples, blind to the known result.

Our study differs from external quality assessment schemes in that these are duplicate results obtained from a large sample of patients across the whole diagnostic range. We strongly recommend that laboratories state an error rate for their measurement on the report; they should participate in external quality assessment schemes and use reference materials regularly to adjust their own internal standards.

P15.09
Efficiency of neurogenetic testing during a 10-year period

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Neurogenetic disorders have low prevalence (<1/10000) in the general population. Specific genetic tests are used to help diagnose neurological diseases depending on the type of mutation. In this study we analysed the diagnostic yield and estimated the costs of negative results of neurogenetic testing.

We performed a retrospective review of the data in the database of the molecular genetic laboratory of the Institute of Medical Genetics, UMC Ljubljana, involving a 10-year period (2000-2010).

Overall, a mutation was identified in 30.9 % of the tested patients (n=443). In patients with muscle weakness (n=205) the mutation detection rate was 38.4 % with an estimated cost of 55,781.95 EUR per negative result. In patients with neuropathies (n=99) the mutation detection rate was 36.1 % with an estimated cost of 53,046.85 EUR per negative test. In patients with chorea (n=100) a positive result was obtained in 42.5 % (22,889.25 EUR per negative result). The diagnostic yield in patients with congenital malformations was 8.9 %; the estimated cost per negative result was 70,931.25 EUR. For ataxias (n=16) the mutation detection rate was 11.9 %; the estimated cost of negative result was 36,071.85 EUR.

The diagnostic yield and costs per negative results varied among the different diagnostic groups, reflecting utilization guidelines and selection criteria for neurogenetic testing. The results of this analysis may assist the clinician when weighing the decision whether to test or not. Guidelines including new testing strategies would contribute to appropriate and cost-effective use of molecular genetic testing in general.

P15.10
External quality assessment schemes for molecular testing on blood spots

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Newborn screening is performed in many countries by analysing dried blood spots to detect abnormal levels of chemicals as indicators of inherited blood and metabolic disorders. Confirmation of clinical diagnosis is often provided by molecular testing and DNA analysis is part of the cystic fibrosis (CF) and Medium chain acyl-CoA dehydrogenase deficiency (MCADD) screening protocols of several newborn screening programmes in Europe.

UK NEQAS for Molecular Genetics is an accredited, self-funding, not-for-profit organization providing external quality assessment schemes (EQAs) for a wide range of molecular disorders since 1991. The scheme has offered "CF molecular testing on blood spots EQA" for the last five years and introduced "MCADD molecular testing for the common ACADM mutation c.985A>G on blood spots EQA" in April 2010. Three blood spot samples are distributed 4 times a year and participants are required to analyse them according to their routine protocol and report the genotyping results. These are assessed for genotyping accuracy. To reflect the laboratory testing, the "MCADD extended mutation screening on blood spots pilot EQA" was introduced last year.

To further enhance these EQA schemes the development of artificial blood spots has been initiated. Using simulated EQA material will enable the distribution of samples with mutations/polymorphisms with the potential to cause interference during analysis or interpretation. The laboratories thereby would be provided with a measure of the robustness of their testing protocol and would be assured of the standard of their service.

P15.11
A quality management plan in a genetic testing laboratory in Romania

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High quality genetic testing is very important for proper diagnosis and patient management.To provide the highest standard of analysis,strict laboratory assurance procedures must be followed.

In this study we developed a quality management plan that would enable us to assure the highest quality standards for genetic testing laboratories.We choosed ISO15189:2007 because it was the more appropriate standard according to our activities.

We nominated a member of the laboratory team to coordinate the plan,but involvement of all laboratory team to implement different parts of the standard for the laboratory is very important.A detailed quality management plan will allow identification of possible deficiencies in laboratory.

It is necessary that laboratory services include an effective labelling(so that the specimens are easily tracked) and adequate physical storage,in addition to the consideration of safety and ethics in genetic laboratory work.Laboratory technicians are responsible for the good quality of the patient's samples and sent for testing to laboratory.

The quality of the primary sample(s),to be collected using appropiate materials in a standardized way,represent the basic factor that affect the quality of performance and delivery of testing services,the adequacy of oversight and quality assurance mechanism.

The most significant percentage of errors that occurs in the laboratory takes place at the preanalytical phase.That phase encompasses test selection,ordering and specimen collection,processing,handling,and delivery to the testing site.

In conclusion,quality indicators reduces errors related to test selection and requests,specimen submission,test performance,and reporting and interpretation of results,leading to improved use of genetic laboratory services, including better health outcome for patients.

P15.12
Quantitative comparison of the sensitivity NASBA-ELISA and RT-PCR-ELISA for measurement of the BCR-ABL genes fusion transcript in CML patients

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Chronic myeloid leukemia is characterized by neoplastic overproduction of myelocytes and neutrophils. Detection of cells carrying BCR/ABL fusion is extremely important in monitoring response to treatment, remission and relapse in CML patients. Among the molecular diagnostic techniques used amplification of BCR/ABL fusion transcript , RT-PCR is regarded to be most sensitive.

In this experimental study, we compared RT-PCR and NASBA techniques to determine quantitatively the number of bcr/abl transcripts. In order to make an accurate comparison, the sequences of primers and probes used in both techniques were the same. Fusion transcripts were synthesized and RNA was extracted from K562 leukemic cell line. A serial dilution of both fusion transcript and RNA was prepared; then sensitivity of both techniques was determined. RT-PCR and NASBA reaction products were labelled using equal ratios of DIG-11-dUTP and DIG-11-UTP respectively. Following denaturation , hybridization reactions were carried out with specific probes. The products were incubated in streptavidin coated microplates. Then, the plates were washed, anti-DIG conjugated to peroxidase added and using ATBS as substrate, enzymatic activity was determined by absorption at 405 nm. The our results showed that specificity between two techniques is equal but RT-PCR-ELISA sensitivity is about a 100-fold more than of NASBA-ELISA. Also, leukemia cell detection rate in sample by RT-PCR-ELISA and NASBA-ELISA is 4 and 400 cells, respectively.

Interpretation and Conclusions: Therefore, despite the NASBA technique have not need to thermal cycler PCR but have a low sensitivity than RT-PCR and it is not suitable method for quantitative assessment.

P15.13
Thrombophilic gene mutations and pulmonary thromboembolism in Iranian patients

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Venous thromboembolism (VTE) is a common vascular disease that includes deep venous thrombosis and pulmonary thromboembolism. It is one of the leading causes of mortality across the world; with an annual incidence range from 1-5 cases per 1000 individuals. Various investigations examined the prevalence of thrombophilic gene mutations in patients who have suffered from a pulmonary thromboembolism. We aimed to determine the association of specific inherited thrombophilias with pulmonary thromboembolisms. A total of 85 subjects were included in this study: 53 patients who had been affected with a pulmonary thromboembolism and 32 healthy controls in the North West of Iran. Total genomic DNA was extracted from 2 ml EDTA anti-coagulated blood samples by a standard salting-out method and amplified by PCR using gene-specific primers. Amplification Refractory Mutation System (ARMS-PCR) method was used in the identification of PAI-1 (-675 I/D, 5G/4G), Factor XIII (Val34Leu), Beta-fibrinogen (-455G/A), Factor VII (Gln353Arg), Glycoprotein Ia (807C/T) and tPA (intron h D/I) gene mutations. Our data indicates that there were statistically significant differences in PTE patients compared with controls, when studying the frequency of these specific gene mutations. In conclusion, we propose that compound thromboplilic gene mutations rather than a specific gene mutation can be a risk factor for the occurrence of pulmonary thromboembolisms; however, further studies on a larger scale are required in order to gain a better understanding of the role of these gene mutations in pulmonary thromboembolisms.

P15.14
Whole genome amplification in large biobanks

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Biobanks are a key resource for unravelling the molecular basis of diseases and the identification of new targets for disease therapy. One challenge of biobanks is to maintain huge collections of limited samples sizes for future studies. In consequence, there is need for a high quality amplification of limited amounts of nucleic acids. In this project we focused on the standardization and qualification tools of whole genome amplification (WGA) in the context of biobanks. A new simple quantitative PCR based test system was developed, which enables the standardization of the complete whole genome amplification process including (1) the quality and quantity of the template DNA and (2) the resulting WGA products. For validation of the performance, the system was tested in different laboratories using template DNAs of different quality. Consistent results could be achieved in the quality system test of the WGA products reflecting the amount and the fragmentation degree of the initial template DNA. The system was also implemented in the KORA-Biobank with SNP genotyping technology. The results of the WGA test system were correlated with the concordance of genome wide SNP genotypes from genomic template DNA and from

the corresponding WGA products. Based on this result, a general standardized protocol for WGA in biobanking will be developed.
Furthermore, the concept of this project is to make the WGA solution available to other national and international biobanks. The development of the proposed, innovative and specialized tools and customized solutions will help to expand and improve the used of biobanks.

P15.15
Microsatellite markers and multiplex ligation-dependent probe amplification (MLPA): Diagnosis tests for Williams-Beuren syndrome

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Williams-Beuren syndrome (WBS; OMIM 194050) is caused by a hemizygous contiguous gene microdeletion at 7q11.23. Supravalvular aortic stenosis (SVAS), mental retardation, overfriendliness, and ocular and renal abnormalities comprise typical symptoms in WBS. Although fluorescence *in situ* hybridization (FISH) is considered the gold standard technique and the microsatellite DNA markers provide efficient confirmation, Multiplex ligation-dependent probe amplification (MLPA) could also be used. MLPA is a new technique for measuring sequence dosage, allowing large number of samples to be processed simultaneously and thus significantly reducing laboratory work. We assessed the performance of MLPA for the detection of microdeletions by comparing the results with those generated using microsatellite markers. We studied 77 patients with clinical diagnosis of WBS using five microsatellite markers: D7S1870, D7S489, D7S613, D7S2476, D7S489_A and commercial MLPA kit (SALSA P029). A total number of 77 patients were tested. Using five markers together, the result was informative in all patients. The microdeletion was present in 64 (83.1%) patients and absent in 13 (16.9%) patients. Maternal deletions were found in 56.3% of patients and paternal deletions in 43.7% of patients. The size of deletions was 1.55 Mb in 57/64 patients (89.1%) and 1.84 Mb in 7/64 patients (10.9%). Our results show a high degree of concordance between MLPA and markers assays for all samples tested. We conclude that even though the microsatellite markers were important for determine the parental origin, MLPA is a highly sensitive, quick and cost-effective alternative and can provide helpful information for clinical diagnose of WBS.

P15.16
A robust measure of workload for molecular diagnostic laboratories

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Recognising a longstanding need for a robust workload measure for diagnostic molecular genetics a group from the CMGS and UKGTN collaborated to devise such a system.
The initial premise for this work was that the measure should be transparent and flexible to allow comparisons between laboratories and to take account of developing technologies. The unit chosen for the measure was the final diagnostic report which is easily counted. As reports vary in complexity a weighting system was required. The unit chosen to weight the reports was ‘an amplicon’ which is again easily counted. The weights were then assigned to a band from A to G. The basic unit, Band A, is sample reception and DNA extraction with a weight of one. Band G has a weight of 40.
This system was piloted in 6 UK laboratories. The laboratories varied in size and testing repertoire to ensure a broad base for the trial.
Work is ongoing to align cytogenetic workload units with these molecular units and to validate the measure by comparing workload assigned to the external quality assessment schemes of UK NEQAS. This allows comparison of many laboratories testing the same cases. The mechanics of the system will be described in detail.

P15.17
Automated melting analysis for quality assessment of high resolution scanning data of multi-exon genes

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High resolution melting analysis for scanning multi-exon genes is emerging as the most cost effective and rapid method to identify sequence variants. A microfluidic liquid handler was used to prepare pre-coated 96-well PCR plates with M13-tailed exon specific primers. PCR was done in 96-well plates followed by high resolution melting analysis on the LightScanner. However, analysis of the data can be a bottleneck in the process. Therefore, an automated analysis method for high resolution melting was developed to scan multiple exons of genes and assess the quality of the data generated. A custom program was developed to appropriately set normalization and overlay parameters, and calculate a Curve Spread value that provides an estimate of the degree of variability among clustered melting curves. The Curve Spread allows quality control of primer plates and master mix lots, and provides a standard metric to identify variant sequences as melting curves that do not cluster well with wild type samples. A higher Curve Spread value reveals a greater degree of variation and allows unknown samples containing variants to be easily identified. This software has been applied to the analysis of 70 clinical specimens suspected of having mutations in *CYBB*, variants of which are the major cause of X-linked chronic granulomatous disease. Standardized analysis eliminates user bias and automatically provides a Curve Spread value that can be used as a guide for judging the quality of amplification and to set decision criteria for reflex testing to identify the specific sequence of variants.

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