

Abstracts of ESHG Plenary Sessions

PL1.1 Peopling of the New World high-Arctic: A Genetic Perspective

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Distinct cultural waves swept through the New World high-Arctic (Canada and Greenland), leaving behind well-preserved material and biological traces in the permafrost. This talk will focus on a major paleogenetic endeavor aimed at determining the genetic signatures of the three ancient high-Arctic cultures - Saqqaq, Dorset and Thule - and ascertaining any genetic relationships between them by analyzing bone, hair and teeth samples from individuals excavated from sites across the Canadian Arctic and Greenland. Current work constitutes the use of state-of-the-art high throughput sequencing to identify genome-wide markers that would help resolve the phylogenetic relationships of the Saqqaq, Dorset and Thule with respect to each other as well as to modern Inuit and Native American populations. Results from this analysis will help disentangle issues surrounding the origins of the first humans in the region, the timing of these migrations, and provide some perspective on the extent to which they have individually contributed to the genetic history of the New World Arctic.

PL1.2 Monogenic diabetes: The success of molecular genetics for improved diagnosis and treatment

P. Njølstad:

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Monogenic diabetes results from mutation in a single gene primarily affecting pancreatic beta or acinar cell function. The prevalence is 1-2 % of all diabetes. Monogenic diabetes is, however, frequently misdiagnosed as type 1 or type 2 diabetes. Knowledge of the genetic etiology of monogenic diabetes has proved essential for improved treatment, prediction of prognosis, genetic counseling and identification and screening of additional family members.

Monogenic diabetes can be divided in two main groups; neonatal diabetes and maturity-onset diabetes of the young (MODY). Neonatal diabetes is commonly used when diabetes occurs before the age of six months. Mutations in several beta cell genes can cause neonatal diabetes, the most important being KCNJ11 and ABCC8 that encode the Kir6.2 and SUR1 subunits of the ATP-sensitive potassium channel, respectively. Children having a mutation in either of these genes can be treated with sulfonylurea rather than insulin injections and with better glycemic control.

Some ten genes can cause MODY. It is important to diagnose GCK-MODY (MODY2) since this is a mild form for diabetes that seldom requires treatment and in which late-diabetes complications are rare. Two other common forms are due to mutations in the transcription factor genes HNF4A (MODY1) and HNF1A (MODY3). These are clinically nearly indistinguishable and are commonly misdiagnosed as type 1 or type 2 diabetes. Sulfonylurea sensitivity is retained why these forms can successfully be treated with low doses of sulfonylureas. Subjects with mutations in HNF1B (MODY5) or CEL (MODY8) often develop both exocrine and endocrine dysfunction, and hence require both pancreatic enzyme supplement and insulin.

Monogenic diabetes should be suspected in subjects with diabetes and an unusual presentation or development. Most will have a positive family history of diabetes, a primary beta cell dysfunction and be negative for antibodies associated with type 1 diabetes.

PL1.3 A human protein atlas to study human genetics

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No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

PL1.4 Genetics of cancer predisposition

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No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

PL2.1* The Effect of Translocation-Induced Nuclear Re-organization on Gene Expression

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Translocations are known to affect expression of genes at the breakpoints and, in the case of unbalanced translocations, alter gene copy number. However, a comprehensive understanding of the functional impact of this class of variation is lacking. We have studied the effect of balanced chromosomal rearrangements on gene expression by comparing transcriptomes of cell lines from controls and individuals with the t(11;22)(q23;q11) translocation. The number of differentially expressed transcripts between translocation carrying and control cohorts is significantly higher than that observed between control samples alone, suggesting that balanced rearrangements have a greater effect on gene expression than normal variation. Many of the affected genes are located on the derivative chromosome 11. We show that this chromosome is concomitantly altered in its spatial organization, occupying a more central position in the nucleus than its non-rearranged counterpart. Consistently, we observe nuclear repositioning of genes that show differential expression in balanced translocation carriers as compared to controls. The movement of the derivative 11 also results in nuclear repositioning of other, non-translocated chromosomes. Our results are consistent with recent studies that experimentally altered nuclear organization and indicate that nuclear position plays a functional role in regulating the expression of some genes. Our study suggests that chromosomal translocations can result in hitherto unforeseen, large-scale changes in gene expression that are the consequence of alterations in normal chromosome territory positioning. This has implications for the patterns of gene expression change seen during tumorigenesis associated genome instability and during karyotype changes that lead to speciation.

PL2.2* PDZD7 is a modifier of and digenic contributor to retinal disease in Usher syndrome

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Usher syndrome is a genetically heterogeneous recessive disease with hearing loss and retinitis pigmentosa (RP). It frequently presents with unexplained, often intrafamilial, variability of the visual phenotype. Here, we identify PDZD7, encoding a homolog of proteins mutated in Usher subtypes 1C and 2D (USH1C, USH2D). We demonstrate interaction between PDZD7 and two Usher disease proteins, GPR98 (USH2C) and USH2A, and their colocalization in the photoreceptor's connecting cilium region. On a homozygous USH2A mutation background, PDZD7 aggravates RP. Heterozygous PDZD7 mutations

were present with truncating mutations in *USH2A*, *GPR98*, and an unidentified locus. We validated the human genotypes using zebrafish, because visual defects are typically not evident in mouse models of Usher syndrome. Our findings in zebrafish were consistent with digenic inheritance of *PDZD7* and *GPR98*, and with *PDZD7* as a retinal disease modifier in *USH2A* patients. *Pdzd7* knockdown produced an Usher-like phenotype in zebrafish, exacerbated retinal cell death in combination with *ush2a* or *gpr98*, and significantly reduced Gpr98 localization in the region of the photoreceptor connecting cilium. Our data challenge the view of Usher syndrome as a traditional Mendelian disorder. As in Bardet-Biedl syndrome, reclassification of Usher syndrome as an oligogenic disease permits a better understanding of its phenotypic variability.

PL2.3* The identification of 180 genetic loci involved in adult height variation highlights the complex genetic architecture of polygenic traits

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Height is a classic polygenic trait: it has been proposed for nearly 100 years that variants in multiple genes influence height; indeed up to 90% of variation in height is attributable to inherited variation. As part of the Genetic Investigation of ANthropometric Traits (GIANT) Consortium, we performed a meta-analysis of genome-wide association studies of adult height, encompassing 2.8 million single nucleotide polymorphisms (SNPs) and 133,800 individuals of European ancestry from 50 individual studies. We detected 207 distinct loci with a SNP associated with height at $P < 5 \times 10^{-6}$ (versus 5 expected by chance). When we examined the 207 SNPs in a replication dataset of over 51,000 samples, 180 reached a combined $P < 5 \times 10^{-8}$. Many loci have multiple associated variants: conditional analysis identified 19 loci that have additional independent strong associations. Together, the associated variants explain up to 12% of height variation. Using a polygenic model, the explained variance reached a maximum value of 16% when including signals associated at $P < 5 \times 10^{-3}$. The associated loci are strongly enriched for genes known to be mutated in monogenic disorders of skeletal growth ($P < 0.001$), and implicate genes in relevant biological pathways, including hedgehog signaling, TGF-beta signaling, extracellular matrix, and growth hormone signaling. In conclusion, we have identified nearly 200 genetic loci influencing adult height. These loci are enriched for genes in biological pathways relevant to human growth, and support a notion that allelic heterogeneity may be a common feature of polygenic traits.

PL2.4* *De novo* mutations of *SETBP1* cause Schinzel-Giedion syndrome

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Schinzel-Giedion syndrome is characterized by severe mental retardation and characteristic facial features. Most patients die before the age of 10 years. Almost all patients occur sporadically raising the possibility that *de novo* dominant mutations could be the underlying mechanism. The exomes of four affected individuals with Schinzel-Giedion syndrome were sequenced to a mean coverage of 43-fold. On average 21,800 genetic variants were identified per patient. After filtering against known variants and selecting variants affecting the same gene in all patients, only a single candidate gene remained, *SETBP1* which encodes SET binding protein 1. Sanger sequencing confirmed the presence of *SETBP1* mutations in these 4 individuals as well as in 8 additional Schinzel-Giedion syndrome patients. All mutations occurred *de novo*, and two mutations were recurrent: c.2602G>A and c.2612T>C in 4 and 5 patients, respectively. Because all mutations were missense, and clustered to a ultra high conserved 11bp exonic region of *SETBP1*, it is highly likely that they cause disease through either a dominant negative or a gain-of-function effect. In conclusion, our study shows the potential of exome sequencing for disease gene identification by unraveling the first dominant Mendelian disorder. It is highly likely that the mutations cause disease through either a dominant negative or a gain-of-function effect. Exome sequencing is particularly useful for identifying these types of mutations for which no other genomewide approach is applicable. *De novo* mutations may be a frequent cause of frequent sporadic conditions with reduced fecundity such as congenital malformations, mental retardation, and psychiatric disorders.

PL2.5 Complete genome sequencing and analysis of diploid African-American and Mexican-American genomes: implications for personal ancestry reconstruction and multi-ethnic medical genomics

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Understanding the contribution of rare and common genetic variants to disease susceptibility will likely require multi- and trans-ethnic sequencing studies that compare the genomes of many individuals with and without a particular disease. Of particular importance will be accounting for the role of population stratification at fine scales both in terms of genomic and geographic location. Here, we present results from sequencing, assembly, and genomic analysis of two diploid genomes from Phase 3 HapMap sequenced to ~20X coverage using the SOLiD System. The donor individuals are of Mexican and African ancestry and represent the first „admixed“ genomes to be sequenced to high coverage. We demonstrate that genomic sequencing provides finer resolution of „admixture breakpoints“ based on allele frequency estimates from HapMap and the 1000 Genomes Project. For each admixed genome, we use the distribution of admixture breakpoints to infer the personal admixture history of the sample and patterns of genomic diversity to reconstruct the demographic history of European, African, and Native American continental populations. Furthermore, we compare the distribution of functional and putatively neutral genetic variation among 12 sequenced genomes and find that difference in demographic history may account for statistically significant, differences in distributions of synonymous vs. benign, possibly damaging, and probably damaging non-synonymous coding variants. Finally, we use the SOLiD comparative personal genomic data sets and 1000 Genomes

data to quantify the relative proportions of private, rare, and common functional and neutral genetic within and among populations.

PL2.6 A mutation in the 3'UTR of the HDAC6 gene abolishing the post-transcriptional regulation mediated by hsa-miR-433 is linked to a new form of dominant X-linked chondrodysplasia.

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A family with dominant X-linked chondrodysplasia was previously described. The disease locus was ascribed to a 24 Mb interval in Xp11.3-q13.1. We have identified a variant (c.*281A>T) in the 3'UTR of the HDAC6 gene that totally segregates with the disease. The variant is located in the seed sequence of hsa-miR-433. Our data showed that, in MG63 osteosarcoma cells, hsa-miR-433 (miR433) down-regulated both the expression of endogenous HDAC6 and that of an eGFP-reporter mRNA bearing the wild-type 3'UTR of HDAC6. This effect was totally abrogated when the reporter mRNA bore the mutated HDAC6 3'UTR. The HDAC6 protein was found to be over-expressed in thymus from an affected male foetus. Concomitantly, the level of total alpha-tubulin, a target of HDAC6, was found to be increased in the affected foetal thymus, whereas the level of acetylated alpha-tubulin was found to be profoundly decreased. Skin biopsies were obtained from a female patient who presented a striking body asymmetry with hypotrophy of the left limbs. The mutated HDAC6 allele was expressed in 31% of left arm-derived fibroblasts, whereas it was not expressed in the right arm. Overexpression of HDAC6 was observed in left arm-derived fibroblasts. Altogether these results strongly suggest that this HDAC6 3'UTR variant suppressed hsa-miR-433-mediated post-transcriptional regulation causing the overexpression of HDAC6. This variant is likely to constitute the molecular cause of this new form of X-linked chondrodysplasia. This represents to our knowledge the first example of a skeletal disease caused by the loss of a miRNA-mediated post-transcriptional regulation on its target mRNA.

PL2.7 The BRCA gene patent dispute: breaking news from America

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PL3.1 Neurogenetic pathways regulated by FOXP2, a gene implicated in speech and language

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People who carry rare heterozygous mutations disrupting the *FOXP2* gene have problems mastering the complex sequences of mouth movements needed for speech, along with deficits in many aspects of expressive and receptive language. The gene encodes a highly conserved transcription factor that helps regulate development and function of neuronal subpopulations in a wide range of non-speaking vertebrates, although evidence suggests that its role(s) may have been modified during human evolution. It is emphasised that *FOXP2* is not the mythical "gene for speech", but represents one piece of a complex puzzle. I will describe how *FOXP2* can be used as a unique window into key neurogenetic pathways via an array of complementary approaches. For example, using functional genomic screening of human neurons grown in the laboratory, we identified the *CNTNAP2* gene (a member of the neurexin superfamily) as a downstream target

directly regulated by *FOXP2*. Intriguingly, we found that *CNTNAP2* is itself associated with common cases of language impairment; this target has also been implicated in language delays of autistic children. High-throughput screening has enabled us to isolate additional putative targets of *FOXP2*, including multiple genes involved in neurite outgrowth and synaptic plasticity. Moving to animal models of *FOXP2* dysfunction, we have shown that point mutations implicated in human speech deficits yield impaired motor-skill learning in mutant mice. Electrophysiological recording suggests that this may be mediated by altered plasticity of *Foxp2*-expressing circuitry. Together with findings from other model systems, these data indicate that the contributions of *FOXP2* to human speech and language are built on evolutionarily ancient roles in neural circuits involved in sensorimotor integration and motor-skill learning. Overall, this work demonstrates how we can begin to bridge gaps between molecules, neurons and the brain, helping us to build more sophisticated models of the relationships between genes, speech and language.

PL3.2 Mice, chimpanzees and the molecular basis of speech

W. Enard:

Max-Planck-Institute of Evolutionary Anthropology, Leipzig, Germany.

Identifying the genetic changes responsible for the phenotypic differences between humans and their close primate relatives is important from an evolutionary, medical and cultural perspective. The primary challenge facing researchers today, after analyzing the genomic data, is experimentally testing hypotheses concerning the genetic basis for human-specific traits. One of the more prominent hypotheses of this kind states that two amino acid changes in the transcription factor *FOXP2* have been fixed in humans by positive selection due to some effect on speech and language. We have introduced these substitutions into the endogenous *Foxp2* gene of mice. Although these mice are generally healthy, they have qualitatively different ultrasonic vocalizations, decreased exploratory behavior and decreased dopamine concentrations in the brain suggesting that the humanized *Foxp2* allele affects basal ganglia. In the striatum, a part of the basal ganglia affected in humans with a speech deficit due to a non-functional *FOXP2* allele, we find that medium spiny neurons have increased dendrite lengths and increased synaptic plasticity. Since mice carrying one non-functional *Foxp2* allele show opposite effects, this suggests that alterations in cortico-basal ganglia circuits might have been important for the evolution of speech and language in humans.

PL3.3 Evolution of Human Language

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PL5.1 ESHG Award Lecture : DNA fingerprinting and the turbulent genome

A. Jeffreys:

University of Leicester, Department of Genetics, Leicester, United Kingdom.

The accidental development of DNA fingerprinting 25 years ago marked the birth of DNA-based identification. I will discuss the origins and evolution of DNA testing, the creation of major national DNA databases and the extraordinary impact that DNA has had on forensic and legal medicine. I will also discuss how DNA fingerprinting identified some of the most unstable regions in the human genome, allowing us to study human DNA evolution in real time and explore the fundamental processes of mutation and recombination that are the ultimate source of all human DNA variation.

Abstracts of ESHG Concurrent Symposia

S01.1 The cradle of constitutional chromosome rearrangements is the cleavage stage embryo

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We developed several novel tools to genome wide screen for CNVs and SNPs in single cells. When applied to cleavage stage embryos from young fertile couples we discovered, unexpectedly, an extremely high incidence of chromosomal instability, a hallmark of tumorigenesis. Not only mosaicism for whole chromosome aneuploidies and uniparental disomies but also frequent segmental deletions, duplications and amplifications that were reciprocal in sister blastomeres were detected in most cleavage stage embryos implying the occurrence of breakage-fusion-bridge cycles. In addition, we demonstrate the existence of those rearrangements in interphase nuclei. The type of rearrangements observed can likely explain the majority of constitutional rearrangements seen in miscarriages as well as live births such as deletions, duplications, inverted deletions, duplications, ring chromosomes and mosaicism of all of those rearrangements. The high frequency of chromosomal imbalances in cleavage stage embryos make it likely that chromosomal disorders originate post-zygotically.

S01.2 How Common is Somatic Mosaicism for DNA Copy Number Variations (CNVs)?

J. P. Dumanski;

Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden.

DNA Copy Number Variation (CNV) has emerged as the most common form of human inter-individual genetic differences and this is important for basic research in biology/genetics as well as for disease-oriented translational science. We have recently discovered that monozygotic (MZ) twins frequently display within-pair differences in CNV profiles, which indicates the feasibility of studying MZ twins, discordant for established phenotypes in search for disease-causing aberrations. In addition, recent analysis of differentiated human tissues of normal deceased subjects supports the notion that somatic CNV mosaicism is underestimated. We tested multiple tissues from three people for differences in CNV profiles and observed changes, affecting a single organ or one or more tissues of the same person. Our results from MZ twins and CNV differences between normal differentiated human tissues of the same person suggest that humans are commonly affected by mosaicism for stochastic CNVs, which occur in a substantial fraction of normal cells and are detectable by available array-based methods. However, the somatic DNA copy number variation is not well studied.

The work in the group focuses on establishment of "baseline of somatic CNV" (the normal frequency and genomic distribution of somatic CNVs) in phenotypically unselected, healthy, concordant MZ twins and comparisons of different tissues from the same individuals. We also study differences in the CNV distribution and/or frequency in MZ twins discordant for various disease phenotypes in search for new disease-related biomarkers.

S01.3 Genomic Disorders: Mechanisms and assays for CNV associated with neuropsychiatric and other disease traits

J. R. Lupski;

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

Whereas Watson-Crick DNA base pair changes have long been recognized as a mechanism for mutations, rearrangements of the human genome including deletions, duplications, and inversions have been appreciated only more recently as a significant source for human genetic variation. Diseases that result from DNA rearrangements have been referred to as genomic disorders. Structural variation of our genome can be responsible for inherited as well as sporadic traits. Rearrangements associated with genomic disorders can be recurrent, with breakpoint clusters resulting in a common sized deletion/duplication, or nonrecurrent and of different sizes. The analyses of breakpoints in the proximal short arm of chromosome 17 (17p) reveal nonallelic homologous recombination (NAHR) as a major mechanism for recurrent rearrangements, whereas nonhomologous end-joining (NHEJ) can be responsible for many of the non-recurrent rearrangements.

Genome architectural features consisting of low-copy repeats (LCRs), also called segmental duplications, can stimulate and mediate NAHR. There are positional hotspots for the crossovers within the LCRs. We recently elucidated a DNA replication mechanism for nonrecurrent rearrangements that we termed FoSTeS - Fork Stalling and Template Switching. A newer model, microhomology-mediated break-induced replication or MMBIR, provides further molecular mechanistic details and may be operative in all life forms as a means to process one-ended, double-stranded DNA generated by collapsed forks. Rearrangements introduce variation into our genome for selection to act upon and as such serve an evolutionary function analogous to base pair changes. Genomic rearrangements may cause Mendelian diseases and complex traits such as obesity and neurobehavioral phenotypes. The mechanisms by which rearrangements convey phenotypes are diverse and include gene dosage, position effects, unmasking of coding region mutations (cSNPs) or other functional SNPs, creating gain-of-function fusion genes at the breakpoints, and perhaps through effects of transvection. *De novo* genomic rearrangements have been shown to cause both chromosomal and Mendelian disease, as well as sporadic traits, but our understanding of the extent to which genomic rearrangements, gene CNV, and/or gene dosage alterations are responsible for common and complex traits remains rudimentary.

1. Hastings, PJ, Ira, G, Lupski, JR (2009) A microhomology-mediated break-induced replication model for the origin of copy number variation. *PLoS Genetics* 5:1-9 [e100327].
2. Lupski, JR (2009) Genomic disorders ten years on. *Genome Medicine* 1:42.1 - 42.11.
3. Zhang, F, Gu W, Hurles, ME, Lupski, JR (2009) Copy number variation in health, disease, and evolution. *Annual Reviews of Genomics and Human Genetics* 10:451-481.
4. Zhang, F, Carvalho, CMB, Lupski, JR (2009) Complex human chromosomal and genomic rearrangements. *Trends in Genetics* 25:298-307.
5. Hastings PJ, Lupski, JR, Rosenberg, SM, Ira, G (2009) Mechanisms of change in gene copy number. *Nature Reviews Genetics* 10:551-564.
6. Stankiewicz, P. and Lupski, J.R. (2010). Structural variation in the human genome and its role in disease. *Annual Reviews of Medicine* 61:437-455.
7. Carvalho, C.M.B., Zhang, F., Lupski, J.R. (2010). Evolution in health and medicine. Sackler colloquium; Genomic disorders - a window into human gene and genome evolution. *Proc. Natl. Acad. Sci. U.S.A.* 107:1765-1771.

S02.1 Ethical issues in large scale genomics research

T. Caulfield;

Health Law Institute, Law Centre, University of Alberta, Edmonton, AB, Canada.

Large, population based biobanking initiatives are in full swing in many countries throughout the world. These projects are principally motivated by a desire to understand the myriad factors that have an influence on human health, including the role and interaction of genetics and the environment. But despite this current research activity, a wide range of policy issues remain unresolved. In fact, the continued existence of these issues is quite remarkable - especially when one considers that many have been debated for over a decade and that the actual practice of biobanking, and the implementation of policy frameworks, has continued notwithstanding this lack of consensus regarding key research ethics principles.

This talk will focus on two of the most persistent and perplexing of the policy issues associated with biobanks: getting consent and allowing participants to withdraw consent. These are not the only issues associated with biobanks, far from it. But they are the two that have attracted much of the policy attention. In addition, getting and withdrawing consent are fundamental principles in research ethics. Understanding the lack of resolution on these key points seems particularly essential. As such, this talk will primarily focus on the reasons for and ramifications of the lack of consensus, including an exploration of whom in the policy community is forwarding the different position. I will analyze factors relevant to the debate, including the role of public perceptions regarding different consent approaches, the law around "ownership" of samples and health information and the idea that this research is in the "public good". In the end, we will see that none of these factors can resolve the debate - at least in the absence of some fundamental and broadly based change in the norms of consent and withdrawal. Despite this reality, I will argue that appropriate governance strategies that specifically engage the issues associated with consent seem the only way forward.

S02.2 Ethical issues in expanded newborn screening**E. W. Clayton;***Vanderbilt University Center for Biomedical Ethics and Society, Nashville, TN, United States.***Ethical Issues Raised by Expanded Newborn Screening**

Wilson and Jungner argued that newborns should be screened only for serious and well understood disorders that require early intervention of proven efficacy prior to the development of symptoms to avert serious or life-threatening sequelae. In recent years, newborn screening has expanded to include disorders that do not meet these criteria. Many factors have led to this expansion, including the availability of multiplex technologies such as tandem mass spectrometry (MS/MS), parent and provider advocacy, and assertions that the appropriate definition of benefit should be expanded. The technical possibility of performing inexpensive whole genome sequencing of newborns lies in the not-too-distant future. In this talk, I will consider what limits, if any, ought to be placed on the expansion of newborn screening. I will consider specifically the critiques raised in the United States by the President's Council on Bioethics in their report entitled *The Changing Moral Focus of Newborn Screening* (2008) as well as reports of Sweden's experience with newborn screening for alpha-1-antitrypsin in early 1970s.

S02.3 Ethical issues in preimplantation genetic diagnosis**G. Pennings;***Bioethics Institute Ghent, Ghent University, Ghent, Belgium.*

Although prenatal and preimplantation genetic diagnosis are frequently considered as similar, there are two differences that have an enormous impact on the ethical evaluation of the applications: 1) the simultaneous availability of several embryos, and 2) the much larger contribution of the clinician to the parental project. The first difference generates the procreative beneficence principle and the lowering of the indications when in vitro fertilization is indicated for other reasons. Some people label this evolution as a slippery slope because they believe that one adheres or should adhere to the strict medical model. This model is based on full penetrance, extreme severity and invariable expression of the disease. Since there are (almost) no such diseases, it is clear that even the currently accepted practice does not fulfill these conditions. We will illustrate this point by means of two examples: sex selection for diseases with skewed sex ratio and variable sex linked expression and selection of healthy carriers.

In the second part, we will consider some of the ethical problems that are generated by the new evolution of genetic screening by means of microarrays. Testing for all chromosomal abnormalities and hundreds of genetic diseases and susceptibilities will confront us with new questions: how to decide which embryo to replace? How to ascertain informed consent before testing? Should information to the parents be limited and if so according to which principles? In essence, the evolution of new techniques always moves in the direction of higher performance but it may confront us with the fact that in reality more information is not necessarily better.

S03.1 Human induced pluripotent stem cell based *in vitro* modeling of Parkinson's disease**F. Soldner;***Whitehead Institute for Biomedical Research, Cambridge, MA, United States.*

Human embryonic stem cells (hESCs) as well as induced pluripotent stem cells (iPSCs) derived from somatic cells of patients are predicted to become a powerful tool for biomedical research and may provide a source for cell replacement therapies. Although the realization of hESC/iPSC based therapies is still at an early stage of development, the possibility to model human disease *in vitro* could make patient-specific iPSCs immediately valuable. This is particularly relevant for diseases of the central nervous system (CNS) such as Parkinson's disease (PD) which are not always linked to known genetic mutations, where primary neuronal tissue is not available, and *in vitro* or *in vivo* animal models only partially recapitulate the underlying pathophysiology. However, there are many technical challenges in generating and manipulating human pluripotent cells before they can be thought to be faithful models of human disease. Here, I will highlight some of the technical challenges and some emerging solutions:

(1) Generation of reprogramming factor-free iPSCs to minimize or eliminate genetic alterations in the derived iPSC lines. The use of viruses encoding the reprogramming factors represents a major limita-

tion of the current technology since residual transgene expression may alter the biological properties of the resulting iPSCs derivatives or induce malignant transformation. We efficiently derived reprogramming factor-free iPSCs from several patients with PD using Cre-recombinase excisable viruses and subsequently differentiated these cells into dopaminergic neurons, the cell type most affected in PD. Such factor-free iPSCs maintain a pluripotent state and display a global gene expression profile, more closely related to hESCs than to genetically identical iPSCs carrying the transgenes. This is consistent with the possibility that residual transgene expression in virus-carrying iPSCs can affect their molecular and biological characteristics and that factor-free iPSCs therefore represent a more suitable source of cells for modeling of human disease.

(2) Efficient gene targeting strategies to generate markers for differentiation and gene correction. Tracking, accentuating, or accelerating pathological phenotypes in the lab could greatly benefit from cell-type-specific lineage reporters, as well as reliable tools to disrupt, repair, or overexpress genes. However, current techniques of gene targeting are inefficient at best and thus are not routinely used. Here we report the highly efficient targeting of several expressed and silent genes in hESCs and iPSCs using zinc-finger nuclease (ZFN)-mediated genome editing.

S03.2 Modeling and treating human genetic disease with induced pluripotent stem (iPS) cells**A. Raya^{1,2,3};**¹*Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain, ²ICREA, Barcelona, Spain, ³CIBER-BBN, Barcelona, Spain.*

The generation of induced pluripotent stem (iPS) cells by ectopic expression of a defined set of factors has enabled the derivation of patient-specific pluripotent cells and provided valuable experimental platforms to model human disease. Patient-specific iPS cells are also thought to hold great therapeutic potential, although several shortcomings should be addressed before iPS cell technology can be implemented clinically. Here, I will present recent results by our laboratory and others on the usefulness of iPS cells to model human disease, the generation of disease-corrected, patient-specific cells with potential value for cell therapy applications, and novel strategies aimed at the generation of clinically-safe iPS cells.

S03.3 Using stem cells to model and treat neurodegenerative diseases**A. D. Ebert;***University of Wisconsin-Madison, Department of Neurology, Stem Cell and Regenerative Medicine Center, Madison, WI, United States.*

Stem cells provide an important tool in which to study human development and disease. Stem cells that naturally carry a genetic mutation, or those that have been genetically manipulated to over-express disease causing mutations, have provided a way to better understand disease processes and mechanisms for a variety of neurological disorders including Down syndrome and Parkinson's disease. The recent advance in stem cell technology in which embryonic stem cell-like cells can be produced by reprogramming somatic cells (termed induced pluripotent stem cells (iPSCs)) has opened yet another window of opportunity to model and study human diseases. iPSCs can now be derived from a multitude of patient populations for both genetically linked and sporadic disorders, including Huntington's disease, amyotrophic lateral sclerosis, and spinal muscular atrophy. Importantly, these iPSCs can be differentiated into the specific cell types that are affected in these brain and spinal cord diseases. Interestingly, in the case of spinal muscular atrophy, motor neurons derived from patient iPSCs have shown selective vulnerability in the culture dish, suggesting a faithful representation of the human disease process. Not only will iPSCs allow for the examination of mechanisms involved in disease progression, but novel drug compound screening and therapeutic intervention may aid in developing more appropriate treatments for patients with these debilitating diseases.

S04.1 From Galton to GWAS: the genetic architecture of complex traits

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Common complex disease is caused by a combination of multiple genes and environmental effects. Traditionally the genetics of disease has been studied using concepts that refer to the combined effect of all genes (e.g., heritability or sibling risk), for example by studying the recurrence risk or phenotypic correlation of relatives. Genome-wide association studies (GWAS) facilitate the dissection of heritability into individual locus effect. They have been successful in finding many SNPs associated with complex traits and have greatly increased the number of genes where variation is known to affect the trait. However, GWAS have been criticised for not explaining more of the genetic variation that we know exists in the population, and many hypotheses have been put forward to explain the missing heritability. The most plausible explanations are that (i) causal effects are too small to be detected with statistical significance and (ii) causal variants are not well tagged by the SNPs on the commercial arrays, for example because their minor allele frequency (MAF) is lower than genotyped SNPs. Genetic linkage and association analyses are typically implemented as a genome scan, i.e. by generating and testing multiple hypotheses. Such approaches, in particular GWAS based upon SNP markers suffer from a high false negative rate because of the use of stringent false positive thresholds. The use of all GWAS data simultaneously in an estimation rather than hypothesis testing framework is a powerful alternative to hypothesis testing. We show how such whole genome methods can be used to better understand the genetic architecture of complex traits, with applications in height and psychiatric disorders. In particular, we show that using genome-wide marker data can provide unbiased estimates of narrow sense heritability and that GWAS SNP data to estimate additive covariance between 'unrelated' individuals can uncover much more of the genetic variance than methods that rely on hypothesis testing.

S04.2 Developments in the genetics of Multiple Sclerosis - progress at last

S. Sawcer;

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Multiple sclerosis is a disabling autoimmune disease of the central nervous system that affects approximately 2.5 million people worldwide (<http://www.atlasofms.org/>). Little is known about the events that trigger the disease or the factors that govern its highly variable course. Epidemiological studies confirm that genetic factors influence susceptibility but relevant genes have proven difficult to identify. Association with the MHC was established almost 40 years ago but alternate candidate gene studies and whole genome linkage screening were unrevealing. Fortunately the advent of genome wide association studies (GWAS) has revolutionised the genetic analysis of multiple sclerosis. To date 7 GWAS have been completed in the disease and 18 associated variants have been identified. This year the International Multiple Sclerosis Genetics Consortium (IMSGC) and the Wellcome Trust Case Control Consortium (WTCCC) will complete a further, and considerably larger, GWAS involving almost 10,000 patients and 16,000 controls. These new GWAS data will substantially expand the list of associated loci and thereby illuminate pathogenesis.

S04.3 Genome-wide association studies in cancer: sorting out the nuggets of truth

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S05.1 Neocentromeres in human clinical cases

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Hospital, Melbourne, Australia.

The centromere is a highly compacted (and morphologically constricted) structure of the chromosome that is essential for the proper segregation of replicated sister chromatids during cell division. A human centromere typically carries 1-4 Mb of repetitive alpha satellite DNA sequences. Human neocentromeres are fully functional centromeres that are formed ectopically on chromosome arms and are devoid of any alpha satellite DNA. The first case of human neocentromere was described by us on band q25 of a rearranged chromosome 10 in a child with mild speech impediment. To date, over 100 cases involving neocentromere formation have been reported with clinical phenotype ranging from very severe to mild or normal, with some of the cases being directly linked to cancer. In addition to humans, the ability of cells to form neocentromeres has been observed in fly, fungi, and higher plants.

Because of their non-repetitive and fully sequenced nature, neocentromeres are highly amenable to molecular analysis. Their study (in parallel with normal centromeres) has led to a better understanding of: (a) the regulatory requirements of the centromere, providing the best evidence that centromere formation is modulated by epigenetic changes at the chromatin level that can occur independently of the underlying DNA sequences. Our work and those of others have also shown that transcription of some of the underlying DNA sequences plays an important role in centromere formation and function; (b) a novel mechanism of cancer development, where studies have shown that a group of atypical lipomas and well-differentiated liposarcomas characteristically carry oncogenic giant supernumerary ring or rod neochromosomes that are mitotically stabilised by the de novo formation of a neocentromere; and (c) a novel mechanism of evolution, where molecular and phylogenetic evidence have shown centromere repositioning via neocentromere formation to be a powerful driving force in chromosome evolution and speciation.

S05.2 Neocentromeres in *Candida albicans*

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Centromeres are critical for chromosome segregation and genome stability. Neocentromeres, functional kinetocores that appear at ectopic loci, can form when centromere function is lost at the normal locus. In humans, neocentromeres can arise in cells with gross chromosome rearrangements to that rescue an acentric chromosome, but have also been described in otherwise healthy individuals where the centromere appears to have been inactivated. The mechanisms of centromere inheritance and neocentromere formation remain unknown. Using the yeast *Candida albicans*, which has small, regional centromeres, we previously found that disruption of the native centromere in *C. albicans* results in neocentromere formation (Ketel *et al.* 2009 *PLoS Genetics* 5(3):e1000400). These neocentromeres form proximal to the disrupted centromere or at distal loci on the chromosome arms far from the disrupted centromere. Distal neocentromere loci characterized to date share properties of low gene density and flanking repeated DNA sequences. We are also using the *C. albicans* neocentromere model system to characterize the functional properties of neocentromeres such as the ability to bind cohesin proteins and other chromatin components and the ability to be stably maintained under stressful growth conditions. Finally, in a genome-wide analysis of replication timing, we find that centromeres replicate at the earliest time during S-phase and that DNA that acquires a neocentromere also acquires this early DNA replication property. Thus, *C. albicans* is a useful model for studying epigenetic activities involving centromeres, such as the establishment of neocentromeres and the maintenance of functional kinetochores at specific DNA loci.

S05.3 Centromere repositioning in evolution and in humans

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In recent years we have used large panels of BAC clones to track the evolutionary history of chromosomes in primates and in non-primate mammals. This approach has disclosed an unprecedented phenomenon: the "centromere repositioning", that is the movement of the cen-

tromere along the chromosome without marker order variation. Repositioned centromeres are relatively frequent. In macaque, for instance, 9 out of 21 centromeres are evolutionarily new; in donkey at least 5 such neocentromeres originated after its divergence from zebra (less than 1 million years). A related phenomenon (clinical neocentromeres) has been reported in human clinical cases. Clinical neocentromeres are analphoid centromeres that emerge in ectopic chromosomal regions. Usually they stabilize supernumerary acentric chromosome which have detrimental phenotypic consequences. Studies on the evolution of the chromosomes where clustering of neocentromeres were reported (3q, 13q, and 15q for instance) disclosed distinct, intriguing relationships between human clinical neocentromeres and evolutionary neocentromeres. Additionally, examples are now available of centromere repositioning events in humans, disclosed by chance because they do not result in phenotypic anomalies.

S06.1 Disruption of long-distance highly conserved non coding sequences at the SOX9 locus

S. Benko¹, J. Amiel¹, A. Munnich¹, D. R. Fitzpatrick², S. Lyonnet¹;

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One of the key discoveries of vertebrate genome sequencing projects has been the identification of non-coding elements that remained evolutionarily conserved, and thus likely functional. Interestingly, two thirds of them do not correspond to transcribed gene sequences (exons and UTRs); they have been named conserved non-coding sequences (CNCs) and represent a vast amount of DNA (3% of the human genome). Interestingly, enrichment for CNCs has been demonstrated within gene deserts nearest to physically isolated genes known or suspected to be important developmental regulators. It has been suggested that in these cases CNCs may represent regulatory elements (enhancers or suppressors) necessary for the correct spatiotemporal expression of these genes needed for embryonic development, and acting as modular, sometimes combinatorial, tissue-specific enhancers of gene transcription.

In that particular context, we will discuss recent findings from our groups regarding:

- A common non-coding enhancer genomic variant in a highly conserved sequence located in a non-coding region of the *RET* gene, altering the binding of a transcription factor expressed in neural crest cell precursors to the enteric nervous system, which would predispose to Hirschsprung disease.
- More recently, the discovery of long-distance disruption of enhancer CNCs on both side of the SOX9 gene coding sequences in Pierre Robin sequence (PRS), a common orofacial cleft anomaly with mandibular hypoplasia. The existence of a PRS locus at 17q24 was supported by both linkage analysis and mapping of independent translocation breakpoints that cluster 1.06-1.23 Mb upstream of SOX9. Also, microdeletions or point mutation involved CNCs capable of driving mandibular expression in transgenic mouse embryos. Moreover, the pattern of histone modifications associated with both the centromeric and telomeric regions suggests tissue-specific enhancer function. ChIP experiments demonstrated that a mutated or deleted CNC binds endogenous MSX1 protein. In addition, a human CNC mutation both alters MSX1 binding and abrogates enhancer function in a mandibular mesenchymal cell line. Our data, combined with existing evidence from human and animal phenotypes, strongly suggests that the disruption of distant, tissue-specific regulatory elements, required for the normal development of the mandibula, perturbs embryonic expression of SOX9 and accounts for the PRS phenotype.

These observations suggest that the domains to study for genomic alterations, resulting in tissue-specific misregulation of a developmental gene and a subsequent malformation, should be much broader than traditionally investigated. They also results strongly suggest that genomic alteration of highly conserved non-coding elements of the genome, located near to, or at a long distance from, coding sequences of a gene might alter gene expression in a tissue-specific and timing-specific manner. These evolutionarily constrained regions of the genome are under purifying selection for function, and with no protein coding activity, may be disrupted in a modular fashion as many such regulatory elements surround master developmental genes. This

model could be regarded as a novel mutational mechanism causing human congenital malformations, and understanding it will certainly provide a powerful tool in establishing etiology for a broader range of human diseases.

S06.2 Clustered gene co-regulation and enhancer sharing can be modulated by developmentally regulated chromatin loops

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Gene clusters are paradigms to study transcriptional regulation during development. Here, we present a general map of enhancer distribution along the 2 Mb of DNA spanning the *IrxA* cluster produced by means of transgenic *Xenopus*, zebrafish and mouse embryos. Using Chromatin Conformation Capture, we demonstrate that enhancer sharing is widespread within the cluster, which explains the common expression domains of *IrxA* genes in particular tissues and the evolutionary conserved architecture of the cluster. We also identify an insulator and two chromatin loops within the cluster that may help partition it in two independent regulatory domains in certain cell types. We finally show that this topology predicts gene expression in cases where cluster organization has been disrupted during evolution. We conclude that the regulatory constraints imposed by the linear arrangement of clustered genes in the genome can be modulated by developmentally regulated loops that facilitate the formation of gene-specific regulatory landscapes.

S06.3 Far reaching consequences - mechanisms and problems of long range control

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The vast majority of most genomes consists of non-coding sequence with more or less unknown function. This "dark side" of the genome contains regions of diverse composition including sequences that are highly conserved throughout evolution. Some of these so called conserved non-coding elements (CNEs) have been identified as essential regulators of gene expression. CNEs are particularly abundant in "genes deserts" surrounding genes that have important functions during development and may be as far as 1 Mb away from the gene they regulate. Gene regulation is achieved through the binding of transcription factors to the element and subsequent loop formation between the CNE and the gene's promotor. Mutations that interfere with the cis regulatory capacity of these elements can thus be expected to result in altered gene expression in a certain cell type at a given time point. We have been investigating the consequences of CNE-controlled gene regulation and the effect of mutations using cytogenetics and high-resolution array CGH in mouse models and patients. We identified several molecular mechanisms that cause abnormalities in long range control. These include the disconnection of control elements from their target gene by translocations, changes in presumed transcription factor binding sites by point mutations, and altered gene regulation by deletions, and duplications of CNEs. All abnormalities were detected in patients or mice with congenital malformations, i.e. brachydactyly, triphalangeal thumb-polysyndactyly, Laurin-Sandrow syndrome, Cooks syndrome, or syndactyly. We postulate that these conditions are caused by alterations of fine tuning of gene expression which in consequence disturbs dosage-dependent signalling pathways. Due to the fact that this mutation mechanism interferes only with a certain regulatory event and not the entire gene function, the resulting phenotypes are distinct from those associated with mutations in the coding region.

S07.1 The impact of the early social environment on the adult epigenome

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No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

S07.2 Identifying parent of origin effects in the human genome**A. Sharp;***University of Geneva, Department of Genetic Medicine and Development, Geneva, Switzerland.*

No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

S07.3 Using *C. elegans* to study chromatin regulators involved in human disease**I. J. Latorre¹, M. Cheung¹, J. Garrigues², A. Vielle-Canonge¹, T. Takasaki², S. Strome², J. Ahringer¹;**¹*Gurdon Institute, The Wellcome Trust/Cancer Research UK, Cambridge, United Kingdom, ²University of California, Santa Cruz, CA, United States.*

Regulation of chromatin structure plays a central role in transcriptional control. A large number of chromatin regulating enzymes and complexes are known, however, their mechanisms of action are poorly understood. Global chromatin factor mapping and loss of function studies in single-celled yeast have provided important insights, but there is still little information on genome-wide targets and functions in multicellular organisms. Importantly, animals contain many chromatin-regulating complexes not found in yeast, such as the histone deacetylase NuRD chromatin-remodelling complex, and the DRM complex, which includes the tumor suppressor Retinoblastoma. Components of both of these complexes have been implicated in human disease. *C. elegans* has many features that make it well-suited for studies of chromatin regulation. Of particular note are its small well-annotated genome (30X smaller than human), the ease of RNAi, and the rich resource of chromatin mutants for loss of function studies. Importantly, *C. elegans* has a complement of chromatin factors very similar to that of humans, allowing investigations of chromatin function in a multicellular organism. As a step toward understanding the functions of chromatin proteins implicated in disease, we are identifying their patterns of binding in *C. elegans* genome-wide using ChIP-chip and ChIP-seq. In addition, to provide a framework for these studies, we are also generating genome-wide maps of the locations of histones and histone tail modifications.

S08.1 Genomic advances in Schizophrenia**M. J. Owen;***MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University, Cardiff, United Kingdom.*

Recent studies have supported the hypothesis that the high heritability of schizophrenia reflects a combination of relatively common alleles of small effect and some rare alleles with relatively large effects. Genome-wide association studies have identified several risk loci at genome-wide levels of significance as well as evidence for a substantial burden of common risk loci. Moreover these recent findings suggest genetic overlap with bipolar disorder which has traditionally been assumed to be genetically distinct from schizophrenia. Genome-wide studies of at least one class of relatively uncommon variant, submicroscopic chromosomal abnormalities often referred to as copy number variations (CNVs), suggest that these confer high risk of schizophrenia. There is evidence both for an increased burden of large (>100kb) and rare (MAF <1%) CNVs in schizophrenia and that risk is conferred by a number of specific large CNVs (including deletions at 22q11.2, 1q21.1, 15q13.2 and 15q11.2 and duplications of 16p11.2) as well as by deletions of *NRXN1* which encodes the synaptic scaffolding protein neurexin 1. Many of these CNVs have been implicated in autism, mental retardation, epilepsy and other neurodevelopment disorders. The implications of recent findings for the pathogenesis and nosology of schizophrenia and related disorders will be discussed.

S08.2 Genome-Wide Association Studies in Autism Spectrum Disorders**H. Hakonarson;***Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA, United States.*

Autism spectrum disorders (ASDs) represent a large group of childhood neurodevelopmental, neuropsychiatric disorders characterized by restricted and repetitive patterns of interests and behavior, limited verbal communication and impairment of social interaction. Several sources of evidence suggest strong a genetic component in the sus-

ceptibility to ASDs; for instance, there are much higher concordance rates for ASDs in monozygotic twins (92%) than dizygotic twins (10%), while recent estimates for the sibling recurrence risk is greater than 15. Although ASDs are highly heritable disorders, they exhibit heterogeneous clinical symptoms and genetic architecture which have hindered identification of common genetic susceptibility factors. Although previous linkage studies, candidate gene association studies and cytogenetic studies have implicated several chromosomal regions for the presence of autism susceptibility loci they have failed to consistently identify genes or genomic loci that increase risk of ASD presentations. Using the genome wide association study (GWAS) approach, we have recently identified common genetic variants between two cadherin genes (*CDH10* and *CDH9*) as associated with ASDs (Wang et al, *Nature*, 2009), as well as a collection of rare copy number variants in neuronal cell-adhesion genes (Glessner et al, *Nature*, 2009). The discovery cohorts in the GWAS contains 780 families (3,101 subjects) with affected children, and a second cohort of 1,204 affected subjects and 6,491 control subjects, all of whom were genotyped by us in the Children's Hospital of Philadelphia (CHOP), representing the largest ASDs genetics studies ever performed. The results from these studies and our ongoing search for the causal variants and their potential influence on the neuronal cell-adhesion molecules in the pathogenesis of ASDs will be presented.

S08.3 Behavioural genetics in mice**J. Flint;***The Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom.*

No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

S09.1 Prenatal diagnosis of skeletal dysplasias**S. Unger;***Centre for Pediatrics and Adolescent Medicine, Freiburg University Hospital, Freiburg, Germany.*

The delineation of skeletal dysplasias originated and still relies heavily on radiographic findings. Translation of this knowledge to ultrasound based diagnosis is not always evident or simple. Ultrasound provides an indirect image that is often focused on details and it can be difficult to get an overview (a form of prenatal babygram). For genetic counseling purposes it is important to distinguish lethal and non-lethal forms of dysplasia. Certain parameters, such as degree of long bone shortening, chest size relative to abdomen, and presence or absence of hydrops or other anomalies, are key to this interpretation. Few signs are specific (and none are pathognomonic) thus a systematic approach to the skeleton is needed. This allows for the best possible chance of diagnosis but this is not always possible prenatally and even the most experienced centers must often give generalized counseling. This talk will review the most common diagnoses as well as some of the pitfalls of ultrasound diagnosis.

S09.2 What you see depends on what you look at Genetics - Fetal ultrasound perspectives**R. Achiron;***University of Tel Aviv, Tel Hashomer, Israel.*

Fetal medicine is a new evolving profession which requires a multidisciplinary approach to confront with various fetal diseases.

Objective: To review the perspectives of Genetic counselling and Sonographic evaluation in fetuses with abnormalities detected in utero.

Methods: A retrospective survey of stimulating and interesting cases will be presented.

Results: Four fetuses representing the following topics are described:

- 1) New Technology and Pandora Box
- 2) One Sees what One Knows
- 3) What you see is the Tip of the Iceberg
- 4) Not Just Images

Conclusion: In modern obstetrics Genetic and fetal medicine collaboration

is necessary for enhancing diagnosis and promoting accurate management.

S09.3 Use of aCGH in prenatal diagnosis*I. B. Van den Veyver;**Baylor College of Medicine, Obstetrics and Gynecology and Molecular and Human Genetics, Houston, TX, United States.*

Current prenatal cytogenetic diagnosis for chromosomal abnormalities is performed by karyotyping of fetal cells from chorionic villi or amniotic fluid to detect any aneuploidy and structural genomic rearrangements of larger than ~5 Mb. In addition, fluorescence *in situ* hybridization is used for rapid detection of common aneuploidies compatible with live birth or detection of deletion and duplication syndromes for which there is high clinical suspicion because of family history or prenatal ultrasound findings. In contrast, array-based Comparative Genomic Hybridization (aCGH) can survey the entire genome for submicroscopic deletions and duplications, aneuploidy and other unbalanced chromosomal abnormalities. It is now widely used in the genetic evaluation of pediatric patients and is being increasingly applied to prenatal diagnosis where it is already making significant impact. Principles of aCGH and different platforms that can be used for prenatal diagnosis, with their benefits and challenges will be briefly reviewed. Our recent experience of over 600 cases suggests that aCGH-detection of copy number alterations of clinical significance from prenatally obtained samples is as high as 8%, depending on the indication, with more than 2% not otherwise detectable. The challenges of pre- and post-test counseling, cost, and potential for finding copy number changes of unknown significance continue to incite debate about the benefit of widespread use of aCGH for prenatal diagnosis; a large multicenter trial is ongoing in the US to address some of these issues. However, the superior diagnostic power of aCGH, the 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, and the decreasing cost, will likely spur universal acceptance of aCGH as a first-line prenatal diagnostic test for fetal chromosomal abnormalities in the near future.

S10.1 Patients' Organizations' Engagement in War on Rare Genetic Diseases: Scientific Activism and New Forms of Sociality*V. Rabeharisoa;**Ecole Nationale Supérieure des Mines de Paris, Paris, France.*

From the 1980's onwards, both in Europe and North America, an increasing number of patients' organizations actively engage in the collection and circulation of knowledge on their conditions. Patients' organizations concerned with rare genetic diseases offer a striking illustration thereof. Some of them even intervene in research activities and contribute to the shaping of research agenda on their diseases. This communication aims at documenting this phenomenon and highlighting its impact on the governance of knowledge, as well as on the dynamics of patients' organizations movements.

I will first show that patients' organizations engagement in research constitutes a watershed in the history of patients' organizations. They do no longer content to provide social and emotional support to their members and to advocate for their rights. They claim to take part in war on their diseases, alongside specialists. In the area of rare genetic disorders, patients' organizations are particularly assertive. Due to the scarcity of knowledge on their conditions, the limited number of specialists interested in, and the difficulties for making rare genetic diseases a public health issue, patients' organizations soon decided to engage in research in order to foster war on their conditions.

Drawing on various case studies, I will then detail different configurations of patients' scientific activism. In the area of rare genetic diseases, patients' organizations do not only provide financial support to research. They mobilize patients' experience and act as "lay-experts" in the process of knowledge production. They thus nurture specialists' expertise with patients' experience from bench to bedside, and give raise to hybrid forms of knowledge on their diseases.

Finally, I will show that patients' scientific activism does not only transform the very nature of knowledge and research activities. It also impinges on patients' identity and lay ground for new forms of sociality that the American anthropologist Paul Rabinow terms "biosociality". To open the general discussion, I will offer a few examples and I will reflect on the social, political and ethical stakes pertaining this new articulation between genetics and society.

S10.2 Transitions between research and clinical practice: Families' experiences in a gene-hunting study*H. Statham¹, M. Ponder¹, M. P. M. Richards¹, N. Hallowell², L. Raymond³;**¹Centre for Family Research, University of Cambridge, Cambridge, United Kingdom, ²Public Health Sciences, University of Edinburgh, Edinburgh, United Kingdom, ³Dept of Medical Genetics, Cambridge Institute for Medical Research, Cambridge, United Kingdom.*

The GOLD study (Genetics of Learning Disability, a joint project undertaken by the Department of Medical Genetics (University of Cambridge) and The Wellcome Trust Sanger Institute) to identify novel gene mutations on the X chromosome, recruited patients and families if:

- the family had multiple male individuals affected by significant Intellectual Disability
- the pattern of occurrence of the Intellectual Disability suggested an X-linked inheritance pattern
- all genes known to be responsible for Intellectual Disability had been tested for and excluded.

This paper reports some of the findings of complementary research with a subset of families who were part of the GOLD study. The objectives were to explore and document the experiences, beliefs, understandings, attitudes and behaviours of family members while they were participating and afterwards.

Analysis revealed similarities between routine clinical practice and the genetic research in terms of process and desired outcome. Thus, in trying to identify the nature of the Intellectual Disability prior to participation in the GOLD study, the *diagnostic* process would have been to take blood and for the blood to be investigated for known genes. The process when the GOLD research was undertaken would also have been to take blood and to investigate the DNA extracted from the blood, but for as-yet unknown gene mutations. Those undergoing testing in either clinical practice or through research sought a genetic diagnosis for a variety of reasons, the most common of which was to allow informed reproductive choices for themselves and other family members. These similarities raise important issues for clinicians involved with patients moving between clinical practice and research with regards consent processes at the beginning of research and the information and care given to participants at the end of research when they again become patients.

S10.3 Genetics and mental illness: perceptions of affected individuals and their family members*J. Austin;**University of British Columbia, Vancouver, BC, Canada.*

To date, there has been little in the way of systematic effort to provide education about what is known (based on research) about the causes of mental illnesses for affected individuals and their families. However, because understanding cause of an illness is critical to adapting to it, in the absence of being provided with an explanation affected individuals and families create their own explanations, based on their experience. For mental illness, these causal explanations often invoke powerful negative emotions like guilt and shame, and can contribute to feelings of powerlessness over the illness. This presentation will review the potential psychosocial consequences of providing genetic counseling for individuals with severe psychiatric illness and their family members, illustrated with case examples from an ongoing randomized controlled trial.

S11.1 Mechanisms of miRNA-mediated gene silencing*E. Izaurralde;**Max-Planck-Institute for Developmental Biology, Tuebingen, Germany.*

MicroRNAs (miRNAs) are genome-encoded ~22 nucleotide-long RNAs that silence gene expression post-transcriptionally by base pairing with the 3' untranslated regions of target mRNAs. To exert their function, miRNAs associate with Argonaute proteins (AGO) in miRNA-induced silencing complexes (miRISCs), which silence the expression of mRNAs containing partially or fully complementary miRNA-binding sites. In animals, most miRNAs are only partially complementary to their targets. In this case, our group has shown that the AGO proteins are not sufficient to mediate silencing and require interaction with proteins of the GW182 family. We have also shown that AGO-GW182 complexes mediate silencing by promoting translational repression and mRNA deadenylation catalyzed by CAF1-CCR4-NOT, the major cytoplasmic deadenylase complex. Deadenylation decreases transla-

tion efficiency and, in somatic cells, commits the mRNA to decapping and 5'-to-3' exonucleolytic degradation. Our analysis of GW182 protein function has revealed two domains critical for silencing: an N-terminal GW-repeat-containing region conferring binding to AGOs, and a bipartite silencing domain, consisting of Mid and C-terminal regions, which elicits translational repression and degradation of miRNA targets. Exactly how the bipartite silencing domain of GW182 proteins interferes with translation and accelerates deadenylation is not completely understood. We have recently started to address this question by showing that the silencing domains of GW182 interact with the cytoplasmic poly(A)-binding protein 1 (PABPC1), suggesting GW182 proteins are PABP-interacting proteins (Paips) that interfere with the function of PABPC1 in translation and mRNA stabilization.

S11.2 The hidden layer of noncoding RNA in the epigenetic control of human development and cognition

J. S. Mattick;

Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia.

Bioinformatic, genomic and experimental evidence all suggest that the genetic programming of humans and other complex organisms has been misunderstood for the past 50 years, because of the assumption - largely true for the unicellular prokaryotes, but not for multicellular eukaryotes - that most genetic information is transacted by proteins. The human genome specifies the development of an anatomically and cognitively complex individual comprised of 100 trillion cells with hundreds of different and precisely sculptured muscles, bones and organs, including the brain, but which contains only about 20,000 protein-coding genes, similar in number and largely orthologous with those in nematodes that have only 1,000 somatic cells. On the other hand, the extent of non-protein-coding DNA increases with increasing complexity, reaching 98.8% in humans, suggesting that much of the information required to program development may reside in these sequences. Moreover it is now evident the majority of the mammalian genome is transcribed, mainly into non-protein-coding RNAs (ncRNAs), and that there are tens if not hundreds of thousands of long and short RNAs in mammals that show specific expression patterns and subcellular locations. Our studies indicate that these RNAs form a massive hidden network of regulatory information that regulates epigenetic processes and directs the precise patterns of gene expression during growth and development. It also appears that RNA is central to brain development, learning and memory, and that animals, especially primates, have developed sophisticated RNA editing systems to modify hardwired genetic information in response to experience, that in turn can modulate epigenetic memory, some of which may be inherited. Thus RNA may represent the computational engine of the cell and the substrate for epigenome-environment interactions. Moreover, what was dismissed as junk because it was not understood may hold the key to understanding human evolution, development and cognition, as well as our individual differences and susceptibilities to complex diseases.

S11.3 System genetics of non-coding RNA

L. Steinmetz;

EMBL Heidelberg, Heidelberg, Germany.

An unanticipatedly large proportion of eukaryotic genomes is transcribed. By profiling genome-wide transcription at high resolution on both strands of the complete yeast genome we have found hundreds of novel intergenic and antisense non-coding RNA transcripts. By comparing transcriptome profiles across multiple conditions and strains with shuffled genotypes, we have defined an annotated set of differentially expressed non-coding transcripts revealing differences in structure and level. Hundreds of non-coding RNAs appear to arise from an inherent bi-directional transcription from eukaryotic promoters. Many others originate from 3' nucleosome depleted regions of genes and are transcribed antisense to the open reading frame. The arrangement and regulatory patterns of these transcripts suggest mechanisms of how they could be regulated and how they could regulate the expression of genes.

S12.1 What can be learned from a large clinical cohort

S. Bergmann;

Department of Medical Genetics, University of Lausanne, Lausanne, Switzerland.

The *Cohorte Lausannoise* (CoLaus) is a random population sample of more than 6'000 individuals who were genotyped for 500.000 Single Nucleotide Polymorphisms (SNPs) using Affymetrix SNP-microarrays. Besides these genotypic markers also a large number of clinically relevant parameters were measured. Comparing the country of origin of these individual with the projection of their genotypic profile onto the principal components of the entire genotypic dataset revealed an astonishingly close correspondence between genetic and geographic distances. Indeed, a geographical map of Europe arises naturally as an efficient two-dimensional summary of genetic variation in Europeans (see Figure). Whole-genome association studies for height, body-mass-index, serum lipid and calcium concentrations, blood pressure and other clinical phenotypes using classical scans testing one SNP at a time elucidated many loci with highly significant associations, which are promising candidates towards unraveling mechanisms of actions and malfunction. Yet, like in many other studies, together these variants only explain a small fraction of the phenotypic variance, indicating that we still miss a comprehensive picture of: (a) what are the causal variants, (b) what effects are attributed by rare variants and/or copy number variations, (c) what fraction of the variance can be explained by SNP-SNP or SNP-environment interactions, and (d) what are the intrinsic limitations of currently used algorithms in dealing with very large sets of genotypic and phenotypic data, which are partially incomplete or noisy. I will outline our research dealing with these challenges.

S12.2 Human genetics from genes to complex networks

R. Xavier;

Andrew D. Smith, Department of Biology, Boston, MA, United States.

No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

S12.3 Pathway Discovery in Adipocyte Biology Using Epigenomics

E. D. Rosen;

Beth Israel Deaconess Medical Center, Boston, MA, United States.

The epidemic of obesity and Type 2 diabetes has thrust the biology of the adipocyte into the forefront of biomedical research priorities. We are interested in identifying the transcriptional basis by which adipocytes govern their behavior. To this end we have utilized approaches that leverage maps of epigenetic alterations in adipose tissue to predict novel transcriptional pathways. We have generated genome-wide chromatin state maps, PPAR γ and CTCF localization maps and gene expression profiles from both murine and human models of adipogenesis. These data provide unprecedented views of chromatin remodeling during cellular differentiation, and allow identification of thousands of putative pre-adipocyte- and adipocyte-specific cis-regulatory elements based on dynamic chromatin signatures. We find that the specific locations of most such elements differ between the two models, including at orthologous loci with similar expression patterns. Based on sequence analysis and reporter assays, we show that these differences are determined in part by evolutionary turnover of transcription factor motifs in the genome sequences, and that this turnover may be facilitated by the presence of multiple distal regulatory elements at adipogenesis-dependent loci. Finally, we also utilize the close relationship between open chromatin marks and transcription factor motifs to identify and validate several novel regulators of adipogenesis and lipid homeostasis.

S13.1 Can breast cancer mortality be reduced with genetic testing?

P. Hall;

Dept. Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden.

No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

S13.2 Common genetic variants and cancer risks for BRCA1 and BRCA2 mutation carriers

A. C. Antoniou, on behalf of the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA);

Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom.

Several lines of evidence suggest that genetic factors modify cancer risks for *BRCA1* and *BRCA2* mutation carriers. Past studies concentrated on variants in candidate genes thought to be functionally relevant to the diseases. However, these have not been very successful and most studies were too small to provide enough power to detect the modest associations that are likely to be present. Recently, polymorphisms identified through genome-wide association studies of unselected cancer patients and controls have been shown to be associated with cancer risk in large studies by the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA). This approach identified seven genetic variants (in *FGFR2*, *TOX3*, *MAP3K1*, *LSP1*, *5q35*, *5p12* and *SLC4A7*) that are associated with the risk of breast cancer and one variant (in *BNC2*) that is associated with ovarian cancer risk for *BRCA1* and/or *BRCA2* mutation carriers. Differential associations were found between these variants and breast cancer risk for *BRCA1* and *BRCA2* mutation carriers. These are in line with differences observed in the associations between these polymorphisms and different disease subtypes in the general population and suggest that studies of mutation carriers may be useful for identifying genetic variants associated with different disease subtypes in the general population. All polymorphisms appear to interact multiplicatively on breast cancer risk for mutation carriers. Based on the joint genotype distribution of the 7 risk associated SNPs in *BRCA2* mutation carriers, the 5% of *BRCA2* mutation carriers at highest risk were predicted to have a probability of 80-96% of developing the disease by age 80, compared with 42-50% for the 5% of carriers at lowest risk. Such risk differences may be sufficient to influence the clinical management of mutation carriers and suggest that this is may be one of the first clinically useful impact of common, low penetrance variants identified through genome wide association studies.

S13.3 Is breast cancer prognosis Inherited?

H. Nevanlinna;

Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Helsinki, Finland.

No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

S14.1 The 1000 Genomes project

R. Durbin;

Wellcome Trust Genome Campus, Cambridge, United Kingdom.

No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

S14.2 International Cancer Genome Consortium

T. Hudson;

Ontario Institute for Cancer Research, MaRS Centre, Toronto, ON, Canada.

The International Cancer Genome Consortium (ICGC) is coordinating an international-scale research effort to obtain a comprehensive description of genomic, transcriptomic and epigenomic changes in the major forms of cancer. This information will lead to better ways of diagnosing, treating and preventing cancer.

The ICGC was launched in April 2008, with the announcement of the proposed strategies and policies to the international scientific community to enable funding agencies and research groups to plan their participation within the ICGC. As of April 2010, the ICGC has received commitments from funding organizations in Asia, Australia, Europe and North America for the following cancer genome projects: acute myeloid leukemia (United States); breast cancer, multiple subtypes (European Union, France, and the United Kingdom); chronic lymphocytic leukemia (Spain); colon cancer (United States); gastric adenocarcinoma (China); glioblastoma multiforme (United States); hepatocellular carcinoma, alcohol and associated etiologies (France) and viral etiologies (Japan); lung cancer (United States); oral cavity cancer (India); ovarian cancer (Australia and United States); pancreatic adenocarci-

noma (Australia and Canada) and entero-pancreatic endocrine tumors (Italy); pediatric brain cancers (Germany); prostate cancer (Canada and United Kingdom); and renal cancer (European Union and France). Each project is expected to involve specimens (tumor plus normal) from approximately 500 patients. Over time, additional nations and organizations are expected to join the ICGC.

Over the next ten years, the ICGC expects to produce comprehensive catalogues of the full range of genetic mutations involved in 50 types of cancer (i.e. 25,000 cancer and 25,000 germline genomes), with key factors being the ability to detect all mutated cancer genes, data at the level of individual DNA bases, application of common standards for pathology and technology and comparison data from matched, non-tumour tissue. The ICGC's informed consent and ethical oversight policies state that cancer patients enrolled in an ICGC-related study should be informed that their participation is voluntary, that their clinical care will not be affected by their participation and that data obtained from analyses using their samples will be made available to the international research community. ICGC projects use common standards of data collection and analysis. In April 2010, the ICGC launched its data portal at www.icgc.org, with the release of several cancer genome datasets that are freely available to the global research community. In my presentation, I will use the examples of pancreatic cancer genome datasets generated by the Australian and Canadian teams.

S14.3 Next Generation Human Genetics

D. Nickerson;

University of Washington, Department of Genome Sciences, Seattle, WA, United States.

No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

S15.1 Using Simple Cells to Model Complex Diseases

S. L. Lindquist;

Whitehead Institute for Biomedical Research and HHMI, Dept. of Biology, MIT, Cambridge, MA, United States.

It is now clear that an astonishing number of human diseases, especially neurodegenerative diseases, result from basic problems in protein folding. These diseases may appear to have little in common with each other besides their devastating effects on patients and their families. Yet one feature they share is the occurrence of complexes of misfolded, aggregated proteins in affected neurons. For each disease, a different protein is the major constituent of the aggregate: in Parkinson's disease alpha-synuclein, in Alzheimer's disease A β and tau. We have developed simple cellular models these protein folding disorders by over-expressing human disease-associated proteins in yeast. By combining the unique power of yeast genetics with the highly conserved biology of protein homeostasis in all eukaryotes, we use yeast cells as "living test tubes" to investigate the mechanisms of toxicity associated with problems in protein folding, trafficking, and degradation and complement these basic studies with transcriptional analysis and high-throughput chemical and genetic screens for toxicity modifiers. Progress will be presented on models for the misfolding of α -syn and A β .

Supported by HHMI and NIH grants NS038372, NS060957.

S15.2 *C. elegans* models for neurodegenerative diseases

E. Nollen;

Department of Genetics, University Medical Centre Groningen and University of Groningen, Groningen, Netherlands.

Various age-related neurodegenerative diseases, including Parkinson's disease, polyglutamine expansion diseases and Alzheimer's disease, are associated with the accumulation of misfolded proteins in aggregates in the brain. However, how and why these proteins form aggregates and cause disease is still poorly understood. Using the nematode worm *Caenorhabditis elegans* to model these diseases and high-throughput genetic screens, we have identified genes that modify aggregation of the disease proteins and their toxicity. We have recently identified an evolutionarily highly conserved modifier of aggregation, *moag-4*, as a positive regulator of aggregate formation in *C. elegans* models for misfolding diseases. We have shown that *moag-4* drives the formation of compact misfolding intermediates that are required for aggregate formation. We have also shown that loss of *moag-4* pro-

motes longevity in parallel to the core IGF/insulin (IIS) longevity pathway, converging at the IIS transcription factors, *daf-16* and *hsf-1*. Thus, *moag-4* has a dual function as a regulator of protein aggregation and of lifespan in *C. elegans*. *moag-4* represents an unexplored protein quality control pathway and since there are two close human orthologs with conserved functions, our results will open up new avenues for research on aging-related neurodegenerative diseases.

S15.3 The prion-like aspect of Alzheimer's disease

M. Jucker;

Department of Cellular Neurology, Hertie-Institute for Clinical Brain Research, University of Tübingen, Tuebingen, Germany.

Cerebral proteopathy is a unifying term for cerebral neurodegenerative diseases in which aggregated proteins are abnormally deposited in the brain. The hallmark proteopathy is Alzheimer's disease (AD) in which fibrillar amyloid- β (A β) peptide is deposited extracellularly in the form

of parenchymal plaques, and hyperphosphorylated tau intracellularly as neurofibrillary tangles. To understand the mechanisms how abnormal protein processing and aggregation leads to cerebral amyloidosis and tangle formation, cellular dysfunction, and dementia, several transgenic mouse models have been generated. These mouse models have been instrumental to study the induction and spread of the AD lesions and a mechanism reminiscent of prions has been suggested. The observation that A β structural variants can be induced *in vivo* in these mouse models intensifies the search of the agent that drives corruptive protein templating in AD pathogenesis. AD lesions and neurodegeneration likely occur many years before the clinical signs of the disease. Thus, an understanding of the earliest and initial events in the development of AD is crucial for the development of diagnostics and early mechanism-based intervention.

Abstracts of ESHG Educational Sessions

ES1.1 Genetics of Female Ovarian Dysfunction

B. C. J. M. Fauser;

Department of Reproductive Medicine and Gynecology, University Medical Center, Utrecht, The Netherlands

Ovarian function is regulated by a complex endocrine (and paracrine) system involving the central nervous system, pituitary gonadotropins, and ovarian steroids. Numerous factors are involved such as kisspeptin, GnRH, LH, FSH (its receptors and signal transduction pathways) and many enzymes and intermediate steroids (in the cascade of converting cholesterol to estradiol), and finally steroid receptors. Rare, single gene mutations and resulting phenotypes have been described for all steps imaginable. Well known phenotypes include Kallman syndrome, 21 hydroxylase deficiency, FSH receptor defect, McCune Albright. These "experiments of nature" greatly improved our understanding of physiology.

Normal menopause occurs between 40 and 60 yrs of age. The age of menopause between sisters (or between mothers and daughters) is closely linked, suggesting a strong genetic component. Age of menopause is related to preceding fertility and has important implications for subsequent female health. Some candidate genes have been associated with age of menopause and currently additional information is generated through the use of genome wide association studies (GWA) using large numbers of samples. Early menopause seems linked to poor IVF outcomes, as well as low response to ovarian stimulation. Primary ovarian insufficiency (POI) is often associated with a premature exhaustion of the primordial follicle pool. Turner Syndrome is linked with the most severe form of POI. Several regions have been identified on the X-chromosome critical for normal ovarian function, and additional mutations, SNPs and CNVs are currently being described on the X chromosome. Many transgenic animal models have been generated to further explore roles of specific genes in ovarian function.

The current focus of genetic research beyond candidate genes in female reproduction is completely geared towards the elucidation of complex and common benign conditions such as polycystic ovary syndrome (PCOS), endometriosis, uterine fibroids.

ES1.2 Genetic Regulation of Spermatogenesis

S. Repping;

Professor Human Reproductive Biology, Center for Reproductive Medicine, Amsterdam, Netherlands.

No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

ES2.1 Finding your feet in the Genome Database World

C. Béroud;

Université de Montpellier, Montpellier, France.

No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

ES2.2 Browsing Genes and Genomes with Ensembl

B. Overduin;

PANDA Coordination & Outreach, EMBL - European Bioinformatics Institute, Hinxton, Cambridge, United Kingdom.

The Ensembl project provides a comprehensive source of annotation for the human genome, along with other species of biomedical interest. Ensembl automatically annotates genomic sequence and predicts the position of genes, to provide a comprehensive range of sequence features and genome wide gene and protein sets. Ensembl also integrates manually annotated gene structures from external sources where available. Apart from the gene sets, Ensembl contains extensive comparative, variation and regulatory information. A rich variety of links to external databases (e.g. OMIM, dbSNP and the NHGRI GWAS catalogue) helps to make Ensembl a key starting and reference point for studies in genetics and molecular biology. Ensembl data are accessible through an interactive web site (<http://www.ensembl.org>), flat files, the data mining tool BioMart, direct database querying and a Perl API.

In this presentation an overview of the Ensembl project and the BioMart

data mining tool will be given with an emphasis on human data in general and variation data in particular.

ES3.1 Family matters: theory and practice in the communication of genetic information

C. Gaff;

Departments of Paediatrics & Medicine, University of Melbourne, Melbourne, Australia.

Facilitating family communication about genetics is an integral part of genetic counselling practice. Although the research literature predominantly focuses on the communication of test results, families inevitably discuss other aspects of genetic conditions, sometimes deliberately, often as part of their regular social discourse. It is likely that communication relating to inheritance and genetic conditions conforms to the rules and patterns that govern communication generally in families. Insights from the discipline of family communication may therefore assist practitioners work with families. They may also enhance awareness of the practitioner's own communication patterns and expectations. After a brief introduction to genetic counselling practice in this field, participants will be introduced to theories from the discipline of family communication - including Communication Privacy Management and Family Communication Patterns. The relevance of these to genetic counselling will be explored, with case studies used as a basis for discussion. Throughout the workshop, there will be an emphasis on reflective practice.

ES3.2 Families and Genomics: A Biopsychosocial Model for Clinical Practice

J. Rolland;

Department of Psychiatry & Behavioral Neuroscience, The University of Chicago, Chicago, IL, United States.

Groundbreaking advances in genomics are identifying genetic components in most major health and mental health disorders. This poses unprecedented challenges for families. How are family and couple relationships being affected by the ability to peer into their possible health and mental health futures? How can we help families use the knowledge of genetic risk to become more resilient and live life more fully?

Drawing from his recent book, *Individuals, Families, and the New Era of Genetics*, Dr. Rolland will first present an overview of his Family System Genetic Illness model to address the psychosocial challenges of genomic conditions for patients and their families, and to help organize this complex biopsychosocial landscape for clinical practice and research. This model clusters genomic disorders based on key characteristics that define types of disorders with similar patterns of psychosocial demands over time. For disorders in which genetic testing is available, core nonsymptomatic time phases with salient developmental challenges are described pre- and post-testing, including a long-term adaptation phase. The FSGI model builds on Rolland's Family System Illness model, which identifies psychosocial types and phases of chronic disorders after clinical onset. The FSGI model is designed to be flexible and responsive to future discoveries in genomic research. Dr Rolland then addresses core issues and cultural influences in decision-making about genetic testing, communication with partners, children, and other family members, and living with risk information across the life cycle. Other key issues discussed include: privacy vs. right to know of others at risk, belief conflicts, impact on childbearing decisions, multigenerational themes, and behavioral genetics. Guidelines are provided for healthcare and mental health professionals to help families master these complex challenges. Its utility is discussed for research, preventive screening, family assessment, treatment planning, and service delivery in a wide range of healthcare settings.

ES4.1 Alport Syndrome

C. Antignac;

INSERM Division: U 423, Paris, France.

No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

ES4.2 Renal cystic disease**N. V. A. M. Knoers:***Radboud University Nijmegen Medical Centre, Department of Human Genetics 417, Nijmegen, Netherlands.*

Cystic kidney diseases are among the most frequent incurable genetic diseases. They are a clinically and genetically extremely heterogeneous group of disorders, encompassing autosomal dominant, autosomal recessive and X-linked traits, which are all characterized by perturbed tubular architecture leading to the formation of cysts. The most prominent examples are Polycystic kidney disease (PKD) and nephronophthisis (NPHP), which affect both adults and children.

In this educational workshop, I will discuss the clinical and genetic aspects of the different types of PKD and NPHP. I will review studies that link these disorders to disturbed structure and/or function of renal primary cilia. Primary cilia are microtubule-based organelles, protruding from the apical surface of almost all cells in the mammalian body, with an antenna-like sensory function. In recent years primary cilia have been shown to play important roles in embryonic development and tissue homeostasis. The ciliary connection also explains the frequent association of extrarenal symptoms with cystic renal disorders, such as liver fibrosis/cysts, retinal degeneration, brain abnormalities, laterality defects, and others. Several examples of these syndromic forms of cystic kidney disease will be highlighted.

ES5.1 Genetic deafness**M. Bitner-Glindzicz:***Institute of Child Health, Ear Institute, Research Area Molecular, Clinical, London, United Kingdom.*

No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

ES5.2 Non-syndromic and syndromic retinitis pigmentosa**H. Bolz^{1,2}:**¹*Institute of Human Genetics, University of Cologne, Cologne, Germany,*²*Bioscientia Center for Human Genetics, Ingelheim, Germany.*

Retinitis pigmentosa (RP) is characterized by progressive loss of night vision in adolescence and constriction of the visual field that may ultimately lead to tunnel vision. Loss of central vision may occur in later life. Most patients are legally blind by the age of 40 years. RP results from loss of retinal rod and cone photoreceptor cells. Diminished retinal function is detectable by electroretinogram long before night blindness, usually the initial symptom, is noticed by the patient. The appearance of abnormal pigment deposits (so-called bone spicule pigmentation) along with retina atrophy/thinning is the hallmark of RP. RP is the leading cause of visual loss in individuals younger than 60 years, with a prevalence of about 1 in 4000. RP is a major cause of blindness. Non-syndromic RP can have different modes of inheritance: autosomal dominant (ADRP, 30-40%), autosomal recessive (ARRP, 50-60%) and X-linked (XLRP, 5-15%). Most isolated cases probably represent recessive disease. Non-mendelian inheritance patterns, such as digenic inheritance and maternal (mitochondrial) inheritance, exist but are probably rare.

RP genes encode for a variety of proteins, reflecting the functional complexity of the retina and including gene products hitherto considered crucial for life such as splice factors and an enzyme of the Krebs cycle. RP is part of many syndromes. In particular, it can be observed in several so-called ciliopathies, e.g. Usher- (with deafness), Joubert- (a developmental disorder with brain malformations) and Bardet-Biedl syndrome. Some of these retinal ciliopathies bridge monogenic and oligogenic inheritance, and variants in certain genes have been shown to act as modifiers of retinal disease. This presentation will give an overview of RP genes, the spectrum of phenotypes and future developments in therapy and research.

ES6.1 The Face Behind the Syndrome**G. Gillessen-Kaesbach:***Institut für Humangenetik, Universität zu Lübeck, Lübeck, Germany.*

Syndromes are typically diagnosed by a combination of clinical features. In many conditions the facial gestalt is of high diagnostic value. Recognition of syndromes like Cornelia de Lange syndrome, Klinefelter syndrome, Treacher Collins syndrome and many others can be

achieved just by looking at the face.

In this educational session the different anatomical structures of the face are explained and you will learn to recognize facial dysmorphisms of the eyes, nose, mouth, ears etc. In a second part of the course syndromes with a recognizable face will be presented. The variable expression of facial features will be demonstrated by showing the mild and severe end of the spectrum. In addition changing of the face with time will be shown. Finally the participants can test their diagnostic skills in a quiz.

ES7.1 Overgrowth**P. Lapunzina:***Section of Medical Genetics, Hospital Universitario La Paz, Madrid, Spain.*

No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

ES7.2 Undergrowth**A. Rauch:***University of Zurich, Institute of Medical Genetics, Zurich, Switzerland.*

No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

ES8.1 Cystic Fibrosis**B. Kerem:***The Hebrew University of Jerusalem, Dep. Genetics, Jerusalem, Israel.*

The gene responsible for the cystic fibrosis (CF) disease defined as cystic fibrosis transmembrane conductance regulator (CFTR) was cloned 20 years ago. The aim of cloning the "CF gene" was to uncover new knowledge that will help to prevent, detect, diagnose and treat patients suffering from the CF disease. In this educational lecture I will overview and discuss: 1. The impact of cloning the CFTR gene on CF incidence and prevalence. 2. Our understanding of the effect of genetic modifiers on disease severity and 3. The current status of therapeutic approaches based on "CFTR knowledge". In the first part of the lecture I will show that in most countries, but not all, we succeeded in finding the CF causing mutations in the majority of patients and by this generated the required basis for genetic testing. The incidence of new CF live birth has decreased in recent years in several countries while in others no change is found. I will discuss potential factors leading to this difference. In the second part I will discuss the molecular basis for disease variability among different patients and review the effect of genetic modifier on the disease severity. In the third part I will summarize the therapeutic approaches which are being developed based on CFTR knowledge. This will include the current status of gene therapy for CF, activation of non-CFTR chloride channels, and mutation specific therapies. In summary, a trend for preventions is already found in many countries, better tools for CF diagnosis were developed, and therapeutic approaches are being investigated. Overall, 20 years of progress enabled us to develop new hopes for a better future for CF patients around the world.

ES8.2 Immotile cilia**H. Omran:***Universitätsklinikum Münster, Zentrum für Kinderheilkunde und Jugendmedizin, Münster, Germany.*

No abstract received as per date of printing, please check the abstract section on the conference website www.eshg.org/eshg2010 for possible updates.

Abstracts of ESHG Concurrent Sessions

C01.1 Tracing the derivation of embryonic stem cells from the inner cell mass by single cell RNA-Seq analysis

K. Q. Lao¹, F. Tang², C. Barbacioru¹, S. Bao², C. Lee², E. Nordman¹, X. Wang¹, M. A. Surani²;

¹Molecular Cell Biology Division, Life Technologies, Foster City, CA, United States, ²Wellcome Trust/Cancer Research UK Gurdon Institute of Cancer and Developmental Biology, University of Cambridge, Cambridge, United Kingdom. The molecular mechanism underlying the transition from the inner cell mass (ICM) of blastocysts to pluripotent embryonic stem cells (ESC) is not fully understood. This is partly because of the apparent heterogeneity amongst a small group of cells, which poses difficulties in investigating this question. Using single cell analysis, including RNA-Seq transcriptome analysis at the resolution of single cells, we have analysed the dynamic molecular network within individual cells from the ICM outgrowth and the established ESC. This study has identified molecular changes that accompany this transition. Our study shows that key genes that confer the property of self-renewal are up regulated as ICM cells progress to ESC. We also detected very significant global changes of transcript variants from individual genes, amongst which the general metabolism genes are strongly over-represented. Furthermore, there was a global increase in the expression of repressive epigenetic regulators with a concomitant decrease in gene activators. The unique ESC epigenotype may thus be sustained while retaining an inherent plasticity for differentiation. Moreover, changes in microRNAs result in one set that targets early differentiation genes, and the second set targets ESC specific pluripotency genes to maintain a delicate balance between pluripotency and a capacity for rapid differentiation. A similar paradigm may also subvert normal developmental sequence in specific adult cells during the formation of diseased tissues, including cancers.

C01.2* Variation in transcription factor binding among humans

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Differences in gene expression may play a major role in speciation and phenotypic diversity. Although variations in gene expression among individuals have been documented, the origins of these differences are not clear, and studies that directly measure differences in transcription factor binding sites among humans have not been performed. We have examined genome-wide variation in transcription factor binding in different individuals and a chimpanzee using chromatin immunoprecipitation followed by massively-parallel sequencing (ChIP-Seq). The binding sites of RNA Polymerase II (Pol II) as well as a key regulator of immune responses, NFκB, have been mapped in ten lymphoblastoid cell lines derived from individuals of African, European, and Asian ancestry, including a parent-offspring trio. Using a stringent threshold, approximately 7.5% and 25% of the respective NFκB and Pol II binding regions exhibit differences between any two individuals. To understand the underlying basis of the variations, we examined the effect of SNPs and genomic structural variations (SVs) on binding differences among individuals. We find that many binding differences are associated with SNPs and SVs. Comparison of the binding data with gene expression data generated by RNA-Seq revealed that differences in binding often correlate with gene expression differences. Furthermore, comparison of the Pol II human sites with binding sites identified in the chimpanzee suggests a high level of divergence in binding relative to our closest evolutionary neighbor. Our results indicate that many differences in individuals occur at the level of TF binding and provide insight into the genetic events responsible for these differences.

C01.3* Genomewide DNA methylation analysis in neurodegenerative disorders

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DNA methylation is one of the most remarkable events within the epigenetic mechanisms of gene regulation, development and genetic imprinting in vertebrates. Alterations in the methylation pattern of regulatory regions have been linked to several pathologies, such as cancer and neuropsychiatric disorders (including schizophrenia and Rett syndrome). In addition, there is an emergent interest to elucidate the relationship between the ageing process, neurodegenerative diseases and aberrant patterns of methylation. For this purpose, we have compared the DNA methylation profile along seven relevant brain areas between Alzheimer's and Parkinson's disease samples and controls. DNA extracted from prefrontal cortex, amygdala, hippocampus, hypothalamus, pons, substantia nigra and cerebellar vermis, from cases and controls, was bisulphite treated and hybridized on an Illumina Infinium methylation array (HumanMethylation27), covering 27,578 CpG sites located in the regulatory region of 14,475 genes and 110 miRNAs promoters. First, an unsupervised hierarchical analysis was used to detect a methylation pattern dependent on brain area, as previously suggested by Ladd-Acosta et al. (2007). Our data show a clustering of all the cerebellar samples (independent of disease status), confirming the observation made by Ladd-Acosta, although the remaining brain areas did not show such a clear clustering effect. Secondly, we proposed to identify disease and tissue specific methylation changes. We observed methylation differences in genes previously described as related to neurodegenerative disease (APOE, PSEN1, SIRT3, and MAOA/B) or to epigenetic mechanisms, as DNMT1, as well as in novel candidate genes.

C01.4 Dissecting the regulatory network of p63 in p63-related developmental disorders

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The transcription factor p63 is a key factor in ectodermal development. Heterozygous mutations in p63 give rise to seven clinical conditions with autosomal dominant inheritance in human. These conditions are characterized by different combinations of split hand-split foot malformation, cleft lip/palate and ectodermal dysplasia (ED). Numerous p63 target genes have been reported; however, their contributions to the phenotypes in the patients carrying a p63 mutation have remained unclear. To understand the regulatory network of p63 relevant to patient phenotypes, we used a disease model, human primary keratinocytes established from patients with p63 mutations. A combination of genome-wide expression profiling and DNA-binding analysis by ChIP-Seq in primary keratinocytes revealed a subgroup of direct novel target genes relevant to ED syndromes. A number of validated p63 binding sites appear to be directly involved in related genetic disorders (cleft lip/palate and split hand-split foot malformation). These binding sites can be found in promoter regions and introns, but in several instances also at distances up to 500 kb from the predicted target gene. Gene expression controlled by p63 binding sites identified in our study was confirmed functionally in transgene reporter assays in zebrafish and mouse. Our study not only increases the repertoire of p63 target genes but also provides a concrete molecular basis to elucidate the disease mechanism of p63 and p63-related developmental disorders.

C01.5 Next generation sequencing-based mRNA profiling of total blood in a large human cohort

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With rapidly decreasing sequencing cost, sequence-based gene expression profiling becomes an attractive alternative over array-based studies. We report on one of the first sequence-based studies into the inter-individual variability of gene expression levels in total blood (n=104). Despite the high abundance of reticulocyte-derived hemoglo-

bin mRNAs (20-80% of reads), the sequencing depth of 10 ± 2.5 million reads per sample allows for the reliable quantification of mRNAs derived from $\sim 12,000$ genes with an expression level down to 0.3 copies per cell. The amount of hemoglobin transcripts shows a significant inverse correlation with white blood cell counts at the time of sample collection. The absolute nature of the expression levels obtained with next generation sequencing, the high sensitivity of the technology, and the presence of cell type-specific transcripts allow for the accurate estimation of the relative amounts of white blood cells, including those for low abundant basophils and eosinophils. Since differences in blood cell content are a major confounding factor in blood-based expression profiling studies, it is essential to correct for these differences before analyzing expression differences between subjects. Unlike array-based studies, sequence-based studies enable the quantification of allele-specific expression using variants in the mRNA-derived sequence reads. We found that the majority of genes demonstrate preferred expression of one of the two alleles. Furthermore, we observed remarkable inter-individual differences in the preference of one allele over the other. Another factor contributing to the inter-individual differences in gene expression is the preferred expression of specific splicing isoforms and/or use of shorter or longer 3'-UTRs.

C01.6 mRNA-Seq transcriptome analysis of human trisomy 21 using monozygotic twins

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Trisomy 21 (T21) is the most widely studied model phenotype of whole chromosome aneuploidy. It is likely that the majority of the T21 phenotypes are related to alterations of gene expression. Transcriptome sequencing now provides the opportunity to investigate in details the perturbations of gene expression in T21 cells and tissues.

In this study we used fibroblasts derived from a pair of monozygotic twins discordant for T21. For the first time, the use of these samples eliminates the bias of genome variability and thus all transcriptome differences observed are likely to be related to the supernumerary chromosome 21.

The transcriptome (polyA+ mRNA) was studied by RNA-Seq; 29 million 76 bp paired-end reads were generated from each sample. We were able to compare the expression of 285'550 exons and 28'178 genes between the samples. We observed that about 9% of exons are differentially expressed between the two samples (FDR<0.01). As expected, we found that the majority (93%) of chromosome 21 exons are overexpressed in the trisomic twin. Differentially expressed exons and genes, chimeric transcripts, splicing variants and allelic expression imbalances will be presented. We will also compare the data with previous microarray studies in order to investigate which transcriptome differences can be validated by sequencing and truly related to the trisomy 21 per se.

C02.1* Identification of ANKRD11 and ZNF778 as candidate genes for autism and variable cognitive impairment in the novel 16q24.3 microdeletion syndrome

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Introduction: The clinical utilization of array comparative genomic hybridization in the evaluation of patients with intellectual disability has recently led to the discovery of a number of novel microdeletion and microduplication syndromes. Aberrations of chromosome 16q with clinical relevance have rarely been reported. Interstitial deletions re-

stricted to band 16q24.3 have not been reported before

Methods: In this study we aimed to characterize the clinical and molecular features of four patients with *de novo* submicroscopic interstitial 16q24.3 microdeletions ascertained by genome-wide array analysis and to determine the shortest region of overlap (SRO) to identify candidate genes responsible for their overlapping phenotype

Results: Clinical features observed in these patients include facial dysmorphisms comprising prominent forehead, large ears, smooth philtrum, pointed chin and wide mouth, variable cognitive impairment, autism spectrum disorder, structural anomalies of the brain and seizures. The common region of overlap of the deletions is only 90 kb and comprises two known genes, *Ankyrin Repeat Domain 11 (ANKRD11)* and *Zinc Finger 778 (ZNF778)*, and is located approximately 10kb distally to *Cadherin 15 (CDH15)*. This region is not found as a copy number variation in controls.

Discussion: We propose that these patients represent a novel and distinctive microdeletion syndrome, characterized by autism spectrum disorder, variable cognitive impairment, facial dysmorphisms and brain anomalies. We suggest that haploinsufficiency of *ANKRD11* and/or *ZNF778* contribute to this phenotype and speculate that further investigation of non-deletion patients who have features suggestive of this 16q24.3 microdeletion syndrome might uncover other mutations in one or both of these genes.

C02.2 Prader-Willi like phenotype in 2pter deletion: a possible imprinted locus.

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Pure subtelomeric deletion of the short arm of the chromosome 2 is an extremely rare chromosomal anomaly. To date only, 4 patients with a pure 2pter deletion have been reported in the literature. The phenotype of these patients correspond either to a Prader-Willi-like phenotype (severe precocious obesity associated to mental retardation and abnormal behavior in 1 patient) or an Angelman-like phenotype (IUGR, mental retardation, speech delay, microcephaly and seizures in 2 patients). The last patient presented with a MCA/MR phenotype but the 2pter deletion was different and distant from the 3 other patients. In addition, 2 other patients with Prader-Willi-like phenotype and unbalanced translocation leading to a 2pter deletion have been reported in the literature.

Here we report on two novel patients with small pure 2pter deletion and Prader-Willi like phenotype. Cytogenetic studies including FISH using BAC-PAC telomeric probes, BAC-PAC Array-CGH and Array-SNP showed small 2pter deletions estimated to 3.15Mb and 1.96 Mb respectively. Both deletions encompassed the TMEM18 gene. Interestingly, a meta analysis of GWAS for obesity found a significant association with a SNP (rs6548238) near to the TMEM18 gene. In addition, Dong et al suggested that 2pter is a possible human obesity-related imprinted locus. These data suggest that the short arm of the chromosome 2 is an imprinted locus associated with phenotypes similar to those observed at the 15q11.2 imprinted locus. Parental origin of the deletion in our patients is in progress in an attempt to accumulate data in favour of an imprinted locus at 2p25.3.

C02.3 2q11.2 is a highly penetrant susceptibility locus for neurocognitive deficit

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One possible explanation for the 'missing heritability' in 'common' disorders such as autism and schizophrenia is the existence of rare alleles of high penetrance, which escape detection in genome-wide association studies. Recent large-scale studies of autism and schizophrenia have implicated copy number variation in the pathogenesis of these disorders ^{1,2}. Previously, a 1.3 Mb microdeletion flanked by segmental duplications at 2q11.2 was reported in a single patient referred for CNV profiling, but no phenotypic information was provided ³. We now report five patients with microdeletions of similar size at this locus (chr2: ~96.0 to ~97.3 Mb, Hg18). All five patients presented with some form of neurocognitive deficit: four with developmental delay, three with autism or autism-like features, and two with epilepsy. We found one further example of this microdeletion in a schizophrenia case in a publicly available CNV dataset ². No examples of this deletion were identified in ~7,500 controls. Notwithstanding that two of the deletions were inherited from a neurocognitively normal father, (one *de novo*, testing of parental samples in the remaining two in progress), this appears to be a very rare but highly penetrant susceptibility locus for neurocognitive deficit. Six highly homologous (>97%) segmental duplications (SD's), size 2.5-40 Kb, cluster at the breakpoints. The rarity of this microdeletion syndrome may be a consequence of the relatively short length of these SD's.

1. Marshall CR et al. *Am J Hum Genet*. 2008 Feb;82(2):477-88.
2. International Schizophrenia Consortium. *Nature*. 2008 455(7210):237-41.
3. Rudd MK et al. *Hum Mol Genet*. 2009 18(16):2957-62.

C02.4 The multiple phenotypes of the recurrent 593 kb, 16p11.2 rearrangements: regulation of adiposity, language impairment and psychiatric symptoms.

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The 16p11.2 deletion has been associated with childhood-onset developmental disorders, macrocephaly and autism in multiple cohorts, while the reciprocal duplication has been associated with microcephaly, schizophrenia and bipolar disorder. We report an association between this deletion and obesity, regardless of the presence of cognitive or behavioral symptoms. This highly penetrant form of adolescent or adult-onset obesity was initially observed in 31 carriers of the 593kb deletion ascertained for cognitive deficits. Nineteen similar deletions were identified from GWAS data of 16053 individuals from 8 European cohorts. Such deletions were absent from healthy non-obese controls and accounted for 0.7% of morbid obesity cases (body mass index, BMI \geq 40 kg.m⁻² or BMI standard deviation score \geq 4; $p = 6.4 \times 10^{-8}$, OR = 43.0), demonstrating the potential etiological importance of rare variants with strong effects in common disease. These rare variants, which escape detection by GWAS, might account for a substantial fraction of patients

with obesity or other "complex traits". In addition, we hypothesize that clinical symptoms in carriers of the deletion and the duplication represent opposite manifestations of the same pathophysiologic process. In search of these "opposite manifestations" mediated by gene dosage, we are currently characterizing BMI, eating behavior, cognitive and psychiatric phenotypes in carriers of both types of rearrangements. Preliminary data reveals that carriers of the duplication show a trend towards being underweight which may confirm this hypothesis.

C02.5 Mesomelia-synostoses syndrome results from deletion of SULF1 and SLCO5A1 genes at 8q13

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Mesomelia-synostoses syndrome (MSS) or mesomelic dysplasia with acral dysostoses Verloes-David-Pfeiffer type is a rare autosomal dominant disorder characterized by mesomelic limb shortening, acral synostoses and multiple congenital malformations. So far, five patients in four unrelated families have been reported worldwide with MSS. Using whole genome oligonucleotide array CGH, we have identified an interstitial deletion at 8q13 in all patients. The deletions vary from 582 kb to 738 kb in size, but invariably encompass only two genes: *SULF1*, encoding the heparan sulfate 6-O-endosulfatase 1 and *SLCO5A1*, encoding the solute carrier organic anion transporter family member 5A1. *SULF1* acts as a regulator of numerous growth factors in skeletal embryonic development while the function of *SLCO5A1* is yet unknown. Breakpoint sequence analyses performed in two families showed non-recurrent deletions. Our results strongly suggest that haploinsufficiency of *SULF1* contributes to this mesomelic chondrodyplasia, highlighting the critical role of endosulfatase in human skeletal development. As co-deletion of *SULF1* and *SLCO5A1* - which does not result from a low-copy repeats (LCRs)-mediated recombination event - was found in all patients, we suggest that haploinsufficiency of *SULF1* combined with haploinsufficiency of *SLCO5A1* (or the altered expression of a neighbouring gene through a position effect) could be necessary in the pathogenesis of MSS.

C02.6 SHOX duplications are associated with type I Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome

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Purpose: MRKH syndrome is defined as congenital aplasia of the structures derived from the Müllerian ducts in females with a normal 46,XX karyotype and secondary sexual characteristics. MRKH is frequently sporadic, although familial cases with an unknown pattern of inheritance have been described. Isolated (type I) and complex (type II) forms exist. The genetic basis of MRKH is largely unknown. Genetic lesions, including *WNT4* point mutations and genomic imbalances, have been identified in a small number of cases. The aim of the study was to identify possible recurrent sub-microscopic imbalances in a cohort of familial and sporadic MRKH cases. Methods: Multiplex ligation-dependent probe amplification (MLPA) was used to screen the sub-telomeric sequences of all chromosomes in 30 MRKH patients (sporadic n= 27; familial n= 3). Segregation analysis and pyrosequencing were applied to confirm MLPA data from the informative family. Twelve patients with clinical signs of hyperandrogenism were also screened for *WNT4* mutations.

Results: A partial duplication in the Xpter PAR1 region containing the *SHOX* gene was found in five MRKH patients (familial n=3; sporadic n=2). The duplications were not overlapping and *SHOX* was never entirely duplicated. Haplotyping in the informative family revealed that the *SHOX* duplication had been inherited from the normal father and was absent in the two healthy sisters. No *WNT4* mutations were identified in the 12 patients with clinical signs of hyperandrogenism.

Conclusions: *SHOX*, which is known to be responsible for Leri-Weill Dyschondrosteosis and Langer Mesomelic Dysplasia, is associated with both familial and sporadic type I MRKH.

C03.1 In-depth metabolic characterization of genetic loci underlying serum-lipids

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Emerging technologies based on mass spectrometry and nuclear magnetic resonance enable the monitoring of hundreds of small metabolites from tissues or body fluids. Because metabolites change rapidly in response to physiologic perturbations, such metabolite concentrations provide a direct readout of the physiological state in the human body, leading to the discovery of novel proximal biomarkers of disease phenotypes. Furthermore, profiling of metabolites in relevant biological pathways can help elucidate the contribution of genetic variants underlying inherited variation in established risk factors. Among the major risk factors for coronary artery disease (CAD) and myocardial infarction (MI) are serum lipids, including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG). To dissect the effect of published genetic variants influencing serum lipid levels, we profiled 151 metabolites covering a biologically relevant panel of amino acids, sugars, acylcarnitines, and phospholipids in 1,797 participants from the KORA population (Germany) and replicated the results in 1,236 participants of the TwinsUK cohort. By analysing lipid concentrations in conjunction with genetic data and metabolite concentrations (and their ratios), we aim to identify cases where a genetic locus is associated with both a lipid and a metabolite concentration, which would provide new functional information about the underlying biological processes. We report here the initial results of this effort, as well as discussing methodological approaches to metabolite analyses. Among others, we identify associations of *GCKR* (glucokinase (hexokinase 4) regulator) variants with different ratios of plasmalogens and phosphatidylcholines ($P = 3.2 \times 10^{-8}$).

C03.2 Identification of novel obesity loci by analysis of genomic structural variants

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Only a small fraction of the strong genetic contribution to obesity is explained by common variants identified from genome-wide association studies. To explore the contribution to obesity of rare variants with large effect, we are investigating the large genomic structural variants (GSVs) that are routinely found in patients with extreme phenotypes that include obesity as a key feature, and analysing these regions in case-control and population cohorts. Using this approach, we have shown that deletions of ~740kb at chromosome 16p11.2 - initially

identified as associated with obesity in patients ascertained for developmental delay - account for ~0.7% of morbid obesity cases in the general population (Walters *et al* (2010) *Nature* 463:671-675).

We are now investigating other GSV regions, identified from cohorts of patients with extreme obesity phenotypes or with severe early-onset obesity, by carrying out algorithmic analysis of genotyping data from multiple cohorts. For one region, similar GSVs are found in non-obese subjects, calling into question the putative association with obesity; for 4 others, GSVs are not found in our cohorts but we have instead identified haplotype signatures that are associated with (or protective against) obesity; and numerous smaller GSVs have helped to define the limits of putative obesity-associated loci. Of particular note is the identification of further instances of 220kb deletions surrounding *SH2B1* (Bochukova *et al* (2010) *Nature* 463:666-670), exclusively in obese subjects and accounting overall for ~0.5% of severe childhood obesity. Targeted exome sequencing is being undertaken to screen for additional rare causal variants in these regions.

C03.3* Multiple common genetic variants for coeliac disease influencing immune gene expression

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Coeliac disease is a complex, highly heritable trait. In our previous genome-wide association study (GWAS) and its follow-ups, we identified 13 non-HLA genomic risk regions for coeliac disease. Known variants, including HLA variants, explain around 35% of the heritability. We proposed that additional common genetic variants would underlie a further component of coeliac heritability.

We performed a new GWAS in 4,533 coeliac cases and 10,750 controls from 4 populations of European descent. We integrated data from our first GWAS and expanded genomic coverage in the new samples to 523,749 SNPs passing quality controls. We further tested 131 SNPs in an additional cohort of 4,918 cases and 5,684 controls.

Variants from 13 new regions showed genome-wide significance ($P_{\text{combined}} < 5 \times 10^{-8}$), including regions containing *TNFRSF14*, *RUNX3*, *CCR4*, *CD80*, *BACH2*, *THEMIS*, *ZMIZ1*, *ETS1*, *CIITA/SOCS1/CLE-C16A*, *ICOSLG*. A further 13 regions had suggestive association evidence ($10^{-6} < P_{\text{combined}} < 5 \times 10^{-8}$, and/or $P_{\text{follow-up}} < 0.01$). Genes from most of these regions have an immune function, while newly identified associations to the *TNFRSF14*, *RUNX3*, *ETS1* and *THEMIS* genes point to an unknown role for the thymic T cell selection pathway in the pathogenesis of coeliac disease. In a meta-analysis of expression quantitative traits in 1,469 whole blood samples, 20 out of the 38 (52.6%) tested loci had coeliac risk variants correlated ($P < 0.0028$, FDR 5%) with *cis* gene expression.

Here we report multiple new common variants for coeliac disease and show that genetic determination of *cis* gene expression is a major mechanism by which these variants influence coeliac susceptibility.

C03.4* Genetic variation in 22 loci influences QRS complex duration

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QRS complex duration, an ECG measurement reflecting ventricular depolarization, is associated with risk of heart failure, sudden death and mortality. The trait is known to have a heritable component (h^2 ranging from 20 - 40%), however few genes influencing the trait have been identified to date. In the current study, a large-scale fixed-effects inverse variance weighted meta-analysis of GWAS data from 16 studies consisting of >40,000 individuals of European ancestry was performed. More than 2.5 million SNPs were imputed using the Hap-Map reference panel. Genomic control inflation factors were used to correct the test statistics at the individual population level as well as the final meta-analysis results. 612 SNPs achieved genome-wide significance levels ($P < 5e-8$). These SNPs represented 30 independent association signals ($r^2 < 0.05$) in 22 distinct genomic regions, of which only four were previously reported. These loci included genes in relevant cardiac conduction pathways, including sodium-channels (SCN10A and SCN5A), calcium handling (PLN/SLC35F1, STRN/HEATR5B, CASQ2, TKT/CACNA1D, and PRKCA), and transcription factors (NFIA, HAND1, TBX3, TBX5, TBX20, and KLF12), in addition to novel pathways (including kinase inhibition and growth-factor related genes). Multiple SNPs demonstrated pleiotropic effects with other ECG traits reflecting atrial conduction (PR interval) and myocardial repolarization (QT interval). Further, we showed experimentally that SCN10A, a gene in our most significantly associated region, is expressed preferentially in the mouse ventricular conduction system and affects mouse QRS interval duration. These analyses greatly expand our understanding of the genetic basis for variation in QRS duration and provide insights into the biology of cardiac conduction.

C03.5* A genome-wide association scan in Sardinians reveals a novel gene associated with multiple sclerosis

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Multiple sclerosis (MS) is a multi-factorial neuroinflammatory and autoimmune disorder. A primary cause of disability in young adults, it re-

sults from interactions between unknown environmental factors and alleles of many susceptibility loci across the genome. Recent investigations of the genetics of MS have resulted in important advances, driven largely by completion of the first genome-wide association scans (GWAS). To detect additional loci, we performed a GWAS in 882 Sardinian MS cases and 872 controls using 575,678 SNPs, genotyped with the Affymetrix 6.0 chip, that passed quality checks. Using imputation methods and haplotypes available from HapMap II, HapMap III and 1000 Genomes projects, we then imputed 6,031,588 SNPs and tested for association ~6.6 million variants. The strongest observed signal was on the HLA locus ($p=1.4 \times 10^{-20}$), at a SNP tag ($r^2=0.83$) for the HLA-DRB1*0301 allele. We then ranked non-HLA SNPs based on their level of significance and proximity to functional candidate genes, and selected 9 SNPs with p value $< 1 \times 10^{-5}$ for follow-up. Of those, one on chr3q13 was successfully confirmed in an independent sample of 1,775 MS cases and 2,005 controls ($p=9.3 \times 10^{-6}$), yielding to an overall p value of 1.6×10^{-10} ($OR=1.40$, $C.I.=1.27-1.57$). The most associated markers at this locus fall in the promoter of a gene that encodes a negative regulator of adaptive immune responses. Mice deficient for the orthologue are prone to experimental autoimmune encephalomyelitis, the animal model of MS but also spontaneously reject a variety of cancers. Hence, this gene appears critical for maintaining the balance between immune activation and tolerance.

C03.6* Genome-wide association scan reveals major susceptibility locus in IL28B for both chronic Hepatitis C and for treatment failure

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The hepatitis C virus (HCV) induces chronic infection in up to 80% of infected individuals, half of whom do not respond to therapy. We conducted a genome-wide association study to screen for host genetic determinants of HCV persistence and response to therapy. The analysis included 1362 hepatitis C infected individuals (448 of whom are co-infected with HIV): 1015 with chronic hepatitis C and 347 that spontaneously cleared the virus. Responses to pegylated interferon-alpha and ribavirin were assessed in 465 chronic hepatitis C patients. Associations between more than 2.5M single nucleotide polymorphisms (SNPs) and outcomes were computed using multivariate logistic regression. Chronic hepatitis C was found to be associated with SNPs in the IL28B locus, which encodes the antiviral cytokine interferon-lambda-3. The minor allele of the top hit rs8099917

was associated with progression to chronic HCV infection (OR=2.31, CI=1.74-3.06, P=6.07*10-9) explaining almost 3% of the phenotypic variance. The association was observed both in HCV mono-infected (OR=2.49, CI=1.64-3.79, P=1.96*10-5) and HCV/HIV co-infected individuals (OR=2.16, CI=1.47-3.18, P=8.24*10-5). Interestingly, SNP rs8099917 was also associated with failure to respond to therapy (OR=5.19, CI=2.90-9.30, P= 3.11*10-8), with the strongest effects in patients with HCV genotypes 1 or 4, where more than 11% of the variance in response was explained by this genotype alone. Re-sequencing of IL28B identified distinct haplotypes that were associated with the clinical phenotype. The association of the IL28B locus with natural and treatment-associated control of HCV indicates the importance of innate immunity and interferon-lambda-3 in the pathogenesis of HCV infection.

C04.1 Identification of novel deafness genes by homozygosity mapping in Dutch families

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Locus heterogeneity of autosomal recessive nonsyndromic hearing loss (arNSHI) is large. So far about 30 genes have been identified but at least 50 genes await identification. Novel arNSHI loci are generally determined in large consanguineous families and are often large (10-30 Mb). Although next generation sequencing enables the simultaneous sequencing of exons and regulatory regions of all genes within a region, strategies to delimit the critical region and "intelligent" candidate gene selection remain attractive for disease gene identification. Although the majority of the Dutch population is regarded to be mixed there are quite a number of regions with limited migration mainly until about 1950. Therefore, we followed a strategy of homozygosity mapping with high density SNP arrays in 125 patients with putative arNSHI from 77 families to delimited the critical region of known deafness loci and/or identify novel loci. Homozygous regions smaller than 1 Mb were not further investigated. We delimited the critical region for DFNB25 to an 0.8 Mb interval harbouring exons of two genes. Also, we obtained indications for a novel locus, now DFNB84, of 3.2 Mb with 11 known and predicted genes. Mutation analysis of all exons in the DFNB25 interval and selected candidate genes in the novel locus revealed putatively pathogenic mutations in *GRXCR1* and *PTPRQ*. Vestibular dysfunction can be associated with the hearing loss for both genes. Our results indicate that the demographic structure of the Dutch population is favourable for this method which is likely to be the case in more European countries.

C04.2 Brown-Vialetto-Van Laere syndrome, a ponto-bulbar palsy with deafness, is caused by mutation in C20orf54

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Brown-Vialetto-Van Laere syndrome is a rare neurological disorder first reported by Brown in 1894 as familial amyotrophic lateral sclerosis with onset in infancy. Following the reports by Vialetto, in 1936 and Van Laere, in 1966, the name, BVVLS was adopted. The key features are progressive ponto-bulbar palsy and bilateral sensorineural deafness. The disease usually presents with VII, IX, X, XI and XII cranial nerve palsies, which develop in a previously healthy individual. A complex neurological phenotype with a mixed picture of upper and lower motor neuron involvement reminiscent of amyotrophic lateral sclerosis evolves with disease progression. The course is invariably progres-

sive, but the rate of decline is variable within and between families. The observation of recurrences in siblings in some families suggested that the condition was probably autosomal recessive, however the variable age of onset and clinical course raised the possibility that it may be aetiologically heterogeneous. We identified a candidate gene, *C20orf54* by studying a consanguineous family with multiple affected individuals and subsequently demonstrated that mutations in this gene were the cause of disease in other, unrelated families.

C04.3* The microRNA miR-204 is required for vertebrate eye development

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The functional role of specific microRNAs in controlling the morphogenetic and cell differentiation events involved in normal eye development in vertebrates is still largely unknown. Here we show that a single microRNA, miR-204, is capable to regulate multiple aspects of eye development in medaka fish. Targeted ablation of miR-204 function by morpholino injections in medaka determined a severe eye phenotype characterized by microphthalmia, aberrant lens formation, incorrect retinal cell differentiation and coloboma. Through a variety of in vitro and in vivo approaches, we found that Meis2 is a key target of miR-204 and plays a pivotal role in the generation of this phenotype via the regulation of the Pax6 pathway. These data demonstrate for the first time that a specific microRNA is involved in the regulation of basic processes underlying eye development and open new avenues on a better comprehension of the pathogenetic mechanisms underlying eye developmental disorders.

C04.4* Phenotypic modifiers of DJ1

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Parkinson's disease (PD) is a slow progressing neurodegenerative disease with devastating clinical symptoms. Current treatments are only symptomatic which ultimately result in debilitating side effects thus there is an urgent need to develop therapeutics which stop and reverse disease progression. None of the 13 loci implicated in the PD pathogenesis represent viable drug targets at present. In addition these genes have suggested that disruption of a myriad of molecular pathways (e.g. ubiquitin and proteasome, mitochondrial function and protein misfolding) can lead to the degeneration of the substantia nigra. It remains unclear how mutation of these genes and disruption of these pathways lead to PD and thus complicates the process for drug discovery.

To address these issues, we conducted a high content screening to interactors of DJ1, a gene mutated in PD to determine if any of them is capable of affecting DJ1 function. Thus far we have been able to identify several genes which are able to rescue DJ1 deficits on cell viability such as PPP2R2C and PSF and other genes which are able to enhance the loss of DJ1 such as 4E-BP. 4E-BP is a translational inhibitor to which many drugs are available thus the administration of 4E-BP activators, such as rapamycin may protect cells from apoptosis and thus an effective treatment to prevent or delay the onset of disease. Using this approach we have been able to construct a detailed molecular pathway of the proteins that are involved in the function of DJ1 and identify additional therapeutic targets.

C04.5 MIR-135b regulates two transcriptional cofactors, PC4 and Psip1, in the mammalian inner ear, identified using an integrative transcriptomics and proteomic approach

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MicroRNAs (miRNAs) are 17-24 nucleotide-long non-coding RNAs processed from transcripts of endogenous genes that function through the RNA interference (RNAi) pathway. miRNAs regulate gene expression by inducing degradation of mRNA of target genes and by inhib-

iting translation. Their relevance to the inner ear has recently been emphasized by the discovery of miRNA mutations leading to deafness in humans and mice. We integrated a comparative transcriptomic and proteomic analyses and a miRNA screen of early post-natal cochlear and vestibular sensory epithelia derived from mice, with sequence-based predictions, to efficiently identify functional miRNAs and their targets. We identified PC4 and Psip1, two transcriptional cofactors that interact with one another, as targets for miR-135b in the inner ear hair cells. PC4, Activated RNA polymerase II transcription cofactor 4, or Sub1, mediates functional interactions between upstream activators and general transcriptional machinery. Psip1, also known as PC4- and SF-2 interacting protein or Lens epithelium-derived growth factor (Lefgf), is involved in transcriptional regulation of stress related genes, mRNA splicing, and cell survival. In order to prove the interaction between the miRNA and its target proteins the miRNA was silenced or over-expressed in cell line and the protein levels were studied using semi-quantitative western blot analysis. Our current work focuses on how miR-135b regulation affects downstream pathways in the inner ear.

C04.6* Olfactory Expression of Mutant A30P alpha-Synuclein in Conditional Mouse Brain: Implications for Early Stage of Parkinson's Disease

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Growing evidence suggests that cognitive and psychiatric deficits precede motor impairment in Parkinson's disease (PD). In this premotor stage neuropathology is detectable in the olfactory bulb and smell deficiency is found in about 90% of PD patients. To explore the impact and reversibility of early pathological stages, we analyzed conditional transgenic mice, expressing high levels of human [A30P]alpha-synuclein limited to the olfactory bulb. Further, as alpha-synuclein positive olfactory lesions of early PD-stages appears not to advance to non-olfactory cortical areas, which are increasingly affected at later stages, their contribution to disease progression is unknown. Thus, the question raise of whether (i) mutated alpha-synuclein is able to induce olfactory pathology, (ii) these alterations influence non-olfactory brain structures and (iii) neurological dysfunction is reversible after ceasing expression of transgene alpha-synuclein. Thus, we generated and characterized a tet-off conditional mouse model expressing human [A30P]alpha-synuclein in the olfactory bulb. We found that over-expressing of mutated [A30P]alpha-synuclein led to a downregulation of dopamine neurotransmission in the olfactory bulb which was paralleled by hyperactivity and decreased anxiety in these mice. We further detected upregulation of neurotransmitter content in striatum and substantia nigra and mitochondrial dysfunction in non-olfactory brain regions, both of which could be reversed in old-aged mice. Perspective: Using this regulatable transgenic mouse model, we may model and explore in detail impact of olfactory alpha-synucleinopathy on other brain regions; this model is also a useful tool to study early intervention strategies, which may halt or even reverse the underlying disease process in PD.

C05.1 CANT1 mutations in Desbuquois dysplasia are responsible for a defect in proteoglycan synthesis.

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Desbuquois dysplasia is an autosomal recessive chondrodysplasia characterized by short stature, joint laxity, scoliosis and advanced carpal ossification with a delta phalanx. Studying 9 Desbuquois families, we identified 7 distinct mutations in the Calcium-Activated Nucleotidase 1 gene (CANT1) which encodes a soluble UDP-preferring nucleotidase. Among the 7 mutations, 4 were nonsense and 3 were missense mutations, located in the region encoding the 7th nucleotidase conserved region and changing arginine at position 300 in 5/9 families. All children presented with characteristic skeletal manifestations. However, an early death due to cardio-respiratory failure was observed in the 4 children with non sense mutations.

The function of CANT1 is unknown. Using RT-PCR analysis, we observed a specific expression in chondrocytes. We also found electron-dense material within distended rough endoplasmic reticulum in Desbuquois patient fibroblasts. Finally, Desbuquois dysplasia shares phenotypic features with Diastrophic dysplasia and recessive Larsen syndrome, which are both due to a defect in proteoglycan sulfation, the final step of proteoglycan synthesis. To test whether CANT1 deficiency interferes with the availability of UDP-sugars needed for proteoglycan synthesis, fibroblasts from two Desbuquois patients and four controls were double labeled with [³⁵S]sulfate and [³H]glucosamine. Surprisingly, in the patient cells glycosaminoglycan (GAG) synthesis was almost normal under basal conditions when compared to controls, but significant reduced GAG synthesis was observed in presence of β -D-xyloside, a compound that increases GAG synthesis acting as a chain initiator. These data suggest that CANT1 plays a role in proteoglycan metabolism and supports its involvement in the rate of GAG synthesis.

C05.2* Disruption of the Podosome Adaptor Protein TKS4 (SH3PXD2B) causes Frank-Ter Haar Syndrome

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Frank-Ter Haar syndrome (FTHS), also known as Ter Haar syndrome, is an autosomal recessive disorder characterized by skeletal, cardiovascular and eye abnormalities, such as increased intraocular pressure, prominent eyes and hypertelorism. We have conducted homozygosity mapping on patients representing twelve FTHS families. A locus on chromosome 5q35.1 was identified for which patients from

ten families shared homozygosity. For one family, a homozygous deletion mapped exactly to the smallest region of overlapping homozygosity, which contained a single gene, SH3PXD2B. This gene encodes the TKS4 protein, a PX and SH3 domain-containing adaptor protein and Src substrate. This protein was recently shown to be involved in the formation of actin rich membrane protrusions called podosomes or invadopodia, which coordinate pericellular proteolysis with cell migration. Mice lacking Tks4 also show pronounced skeletal, eye and cardiac abnormalities and phenocopied the majority of the defects associated with FTHS. These findings establish a role for TKS4 in FTHS and embryonic development. Mutation analysis revealed five different homozygous mutations in SH3PXD2B in seven FTHS families. No SH3PXD2B mutations were detected in six other FTHS families, demonstrating the genetic heterogeneity of this condition. Interestingly however, dermal fibroblasts from one of the individuals without an SH3PXD2B mutation nevertheless expressed lower levels of the TKS4 protein, suggesting a common mechanism underlying disease causation. This is the first time that a developmental disorder is caused by the defect in podosomes. Further study on the role of podosomes may open a new horizon in identifying the genetic defects in related developmental disorders.

C05.3* *PTHLH* deletion and point mutations are associated with Brachydactyly type E (BDE)

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Brachydactylies are a family of limb malformations characterized by short hands/feet due to aplastic/hypoplastic skeletal elements. Autosomal dominant brachydactyly type E (BDE, MIM 113300) is characterized by shortening of metacarpals/metatarsals and/or phalanges. Here we describe a novel disease gene for BDE in five unrelated families. Initially we detected a microdeletion encompassing *PTHLH*, the gene coding for parathyroid hormone related protein (PTHRP) in a pedigree with BDE, short stature and learning disabilities. PTHRP regulates the balance between chondrocyte proliferation and hypertrophic differentiation during endochondral bone development. *Pthrp-/-* mice show short limbed dwarfism due to premature differentiation of chondrocytes. Therefore we screened a cohort of individuals with BDE and short stature for mutations in *PTHLH*. We identified two missense (p.L44P and p.L60P), a nonstop (p.X178WextX*54), and a nonsense (p.K120X) mutation. Functional testing of the L60P missense mutation in chicken micromass culture showed an earlier differentiation of chondrocytes compared to wildtype which indicates that the missense mutation results in a loss of function.

Since mutations of the primary mediator of PTHRP/PTH receptor signaling, GNAS1, are associated with Albright Hereditary Osteodystrophy (AHO) which includes a skeletal phenotype strikingly similar to the BDE phenotype we conclude that the IHH/PTHRP pathway is of particular importance for cartilage differentiation and growth in the metacarpals. These results support the concept of molecular disease families based on phenotypic similarities and interacting signaling pathways. Taken together, mutations in *PTHLH* result in a specific type of skeletal disease which we suggest to name BDE with short stature, *PTHLH* type.

C05.4 Homozygous inactivating mutations in the NKX3-2 gene result in spondylo-megaepiphyseal-metaphyseal dysplasia

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Spondylo-megaepiphyseal-metaphyseal dysplasia (SMMD) is a rare autosomal recessive skeletal dysplasia with only a few cases reported in the literature. Affected individuals have a disproportionate short stature with a short neck and trunk and relatively long limbs that may show flexion contractures of the distal joints. The most remarkable radiographic features are the delayed and impaired ossification of the vertebral bodies as well as the presence of large epiphyseal ossification centers and wide growth plates in the long tubular bones. Genome wide homozygosity mapping followed by a candidate gene approach resulted in the elucidation of the genetic cause in three new consanguineous families with SMMD. Each proband was homozygous for a different inactivating mutation (c.336_337delGGinsT; c.337dupG; c.104_110delCGCCCG) in *NKX3-2*, a homeobox-containing gene located on chromosome 4p15.33. Expression studies in skin fibroblasts showed partial nonsense mediated decay of the mutant transcripts and upregulation of mutant *NKX3-2* mRNA compared to control, the latter suggesting a disturbed autoregulatory loop. We observed the highest expression of *NKX3-2* in chondrocytes and gut. In contrast to previous reports we were also able to demonstrate *NKX3-2* expression in the brain. Striking similarities were found when comparing the vertebral ossification defects in SMMD patients with those observed in the *Nkx3-2* null mice. Distinguishing features were the asplenia found in the mutant mice and the radiographic abnormalities in the limbs only observed in SMMD patients. This study illustrates that *NKX3-2* plays an important role in endochondral ossification of both the axial and appendicular human skeleton.

C05.5 Copy Number and Sequence Variants in *FREM1* are Associated With an Increased Risk of Isolated Metopic Craniosynostosis in Humans and Mice

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Metopic craniosynostosis (MC), the premature fusion of the frontal bone primordia, results in trigonocephaly and has a frequency of 1:10,000 live births. One form of MC is associated with monosomy for an 8Mb interval of chromosome 9p22.3 (OMIM 158170). Features include mental retardation, trigonocephaly and midface hypoplasia. Overlapping copy number variants (CNVs) have been identified which include the *FREM1* gene. We wished to investigate the role of *FREM1* in more than 100 people with MC ascertained through an international craniofacial consortium. The presence of 9p CNVs was assessed by microarrays and *FREM1* was screened for mutations by sequencing and MLPA. *Frem1* knockout mice were also imaged with microCT to determine if craniofacial anomalies consistent with a human MC phenotype.

Two *de novo* CNVs involving *FREM1* and 3 human *FREM1* alleles (one *de novo*) were identified. The two CNVs interrupt the *FREM1* coding sequence, likely resulting in structural abnormalities of the *FREM1* protein. Evidence that a p.Glu1500Val mutation may be involved in MC includes that it is *de novo* in a single family and is present in a second family with a range of craniofacial abnormalities. Craniofacial imaging of homozygous *Frem1* knock-out mice demonstrated craniofacial deformities consistent with MC. Taken together, these findings account for a significant percentage of a uni-sutural craniosynostosis and are consistent with *FREM1* variants increasing the risk of MC. We recommend that all children with MC should have 9p CNV investigations and consideration be given to *FREM1* mutation analysis.

C05.6 Cranioectodermal dysplasia is a ciliary disorder caused by defects in the IFT122 gene

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Cranioectodermal dysplasia (CED, Sensenbrenner syndrome, OMIM 218330) is characterized by craniofacial, skeletal, and ectodermal abnormalities. Most cases are sporadic, but a few familial ones support an autosomal recessive inheritance pattern.

We collected 13 CED patients from 12 independent families. In two families the patients had consanguineous parents, and in one of these, two siblings were affected, permitting linkage analysis and homozygosity mapping. This revealed a single region of homozygosity with a significant LOD score (3.57) on chromosome 3q21-3q24. By investigating candidate genes from this interval we found a homozygous missense mutation in exon 15 (V->G) of the IFT122 gene that co-segregated with the disease. In addition we identified another homozygous missense change (S->F) in exon 11 if IFT122 in the patient from the second consanguineous family, and found compound heterozygosity for two different IFT122 mutations (a splice site change in intron 6 and a missense change in exon 1) in a sporadic patient. All changes were absent in 340 control chromosomes. The IFT122 protein is a component of the retrograde intraflagellar transport and important for in the assembly and maintenance of eukaryotic cilia. We therefore investigated the primary cilia in patient fibroblasts and found significantly reduced cilia frequency and length in patient cells as compared to controls. We next transiently knocked down ift122 in zebrafish embryos and observed a characteristic ciliary phenotype, confirming that CED is a ciliary disorder and suggesting that the causative mutations in the unresolved cases are most likely to affect primary cilia function as well.

C06.1 Is there an increased risk of congenital malformations after Assisted Reproductive Technologies (ART)? Results of a French cohort composed of 15 162 children

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The aims of this study is to estimate the risk of major or minor congenital malformations and genetic disorders among children born after in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) in 33 French centers registered for ART.

The survey was prospective from 2003 to 2007. The cohort was composed of 15 162 infants recruited from birth; medical data were collected regularly from birth to the age 60 months. Questionnaires were fulfilled by the paediatrician and the parents and 98% of them were exploitable. The prevalence of each malformation identified among this cohort was compared to the data of national registers.

A major congenital malformation was found in 4.24% children (vs 2-3% expected). This higher rate was partly due to an excess of heart diseases (0.45% vs 0.25%) and malformations in the urogenital system [uropathy : 1.25% (vs 0.08-0.65%), hypospadias : 0.37% (vs 0.29%)]. Among the minor malformations, a 5 times higher frequency of angioma was reported with a totally skewed sex ratio (262 girls/103 boys). Surprisingly, the average age for parents of malformed children at the time of the conception was not statically different from the all parents of

this cohort. Genetic disorders were found in 110 children. Six children presented with Beckwith-Wiedemann syndrome (0.04% vs 0.007% expected) and 5 with a retinoblastoma (0.03% vs 0.006%), both diseases influenced by epigenetics.

More investigations will highlight detailed informations about factors impacting on the occurrence of these abnormalities, as parental patterns, indications or ART techniques.

C06.2 The origin of de novo chromosome deletions identified by array CGH

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Among de novo cytogenetically visible chromosome imbalances with non-recurrent breakpoints, there is an overall excess of paternal cases: this effect is genome wide but varies according to the type of abnormality and the chromosomal location. These imbalances are likely to arise meiotically and are not associated with increased parental age. In contrast, while non-recurrent balanced translocations are also predominantly paternal in origin, they display a significantly elevated paternal age suggesting they may arise during mitosis in male germ cells prior to meiosis.

The use of array CGH provides two significant advantages in the study of chromosome abnormalities: it allows the aetiology of very small imbalances to be investigated and defines precisely the size of an imbalance thus giving information on the sequence composition around the breakpoints.

We have investigated the parental origin of 37 deletions identified or characterised by array CGH using the Agilent 44K platform. Twenty seven (73%) were paternal in origin and 10 maternal (27%). Analysis of the breakpoint intervals showed that one of the maternal cases and five of the paternal cases contained segmental duplications suggesting formation by Non-Allelic Homologous Recombination. The average size of the paternal deletions was 7.3Mb compared with 3.1Mb for the maternal cases. These results confirm the parental origin bias, although among deletions below 6Mb the excess of paternal cases was less pronounced.

We will investigate the origin of further cases including duplications and complex abnormalities with multiple breakpoints and also look for any association with increased parental age.

C06.3 Novel insights into the pathogenesis of common aneuploidies using genomic analysis of cell-free amniotic fluid

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Background: As a novel means of identifying pathophysiologic changes in fetuses with common aneuploidies, we characterized developmental gene expression using cell-free mRNA in residual second trimester amniotic fluid (AF) supernatant samples.

Methods: RNA was extracted from AF in fetuses with trisomy 21 [T21] (n=7), trisomy 18 [T18] (n=5), and euploid controls (n=13). cDNA synthesis and biotin labeling were performed prior to hybridization to Affymetrix U133 plus 2.0 arrays. Initial analysis was done using the Affymetrix Gene Chip Microarray Suite 5.0, followed by comparative t-tests and Benjamini-Hochberg adjustment. Differentially-expressed genes were further examined by the Database for Annotation, Visualization, and Integrated Discovery (DAVID) and Ingenuity®.

Results: In T21 we identified 414 differentially-expressed genes vs. controls. Only 5/414 genes mapped to chromosome 21. In T18, only 7/356 differentially-expressed genes were on chromosome 18. Heat map and functional analyses of genes not on 18 or 21 showed a consistent pattern unique for each aneuploidy that significantly differed from euploidy. Only 6 differentially-expressed transcripts were common to both aneuploidies. T21 samples showed significant oxidative stress and disruptions in ion transport, G-protein signaling, immune response, and circulatory system function. T18 showed down-regulation of the endocrine system and up-regulation of lipid metabolism.

Conclusions: Our results question the conventional wisdom that the pathophysiology of aneuploidy is due to a gene dosage effect, as the molecular abnormalities observed here are predominantly produced by genes on chromosomes other than 18 or 21. This discovery-driven genomic approach using discarded material suggests new avenues to

further understand abnormal fetal development.

C06.4* Frequencies of 15q11-q13, 7q11.23 and 22q11.2 deletions and duplications in spermatozoa from Prader-Willi syndrome fathers.

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Prader-Willi syndrome (PWS) is a genomic disorder mostly caused by 15q11-q13 deletions (70%) due to meiotic Non-allelic homologous recombination (NAHR) between flanking Low Copy Repeats (LCR). A higher rate of 15q11-q13-deletions and duplications was previously reported in spermatozoa of fathers with PWS affected offspring. The aim of this work was to analyze the frequency of deletions and duplications of other regions with similar features (7q11.23 and 22q11.2) in order to assess whether a higher risk of transmitting other genomic disorders is present in this subjects.

Semen samples from 16 PWS fathers and 10 control donors were processed and analyzed by triple-color FISH as standardized in our laboratory. A customized combination of probes (Abbot Molecular Inc.) was used to assess the target regions allowing the discrimination between normal, deleted and duplicated sperm genotypes. A minimum of 10000 sperm were analyzed per sample and region.

As a whole, higher rates of deletions and duplications were observed for 15q11-q13 (0.9%±0.14) and 22q11.23 region (0.44%±0.09) compared with the control population (0.46%±0.07 and 0.27%±0.05 respectively) (Mann-Whitney test; P<0.05). Interestingly, all individuals with significant increases of 7q11.23 and 22q11.2 had also been reported to have increases of 15q11q13-deletions and duplications. Moreover, a significant correlation were observed between the frequencies of deletions and duplications of 15q11-q13 and 7q11.23 regions (r=0.87; P=0.02) and between 15q11-q13 and 22q11.2 regions (r=0.89; P=0.002).

Results suggest that other factors apart from architectural features in these regions could participate in the increases of NAHR events.

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C06.5* Genome-wide single cell array analysis for preimplantation genetic diagnosis of a complex chromosomal rearrangement carrier

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Patients carrying a complex chromosomal rearrangement (CCR) have an increased risk for chromosomally unbalanced conceptions, which either do not implant, are spontaneously lost during gestation or lead to severely handicapped children. Preimplantation genetic diagnosis (PGD) can select against these embryos carrying unbalanced rearrangements, however only 7 loci can be screened and an indication-specific preparation is needed using the standard FISH-technique. Microarray technology, on the contrary, can detect imbalances as small as 10 Mb genome-wide at the single cell level. We performed for the first time microarray-based PGD for a CCR-carrier with karyotype 46,XY,ins(3;2)(p23; q23q14.2),t(6;14)(p12.2; q13). In concurrence with the ethical committee, only the copy number status of the chromosomes involved in the CCR were diagnosed.

Two PGD cycles with single embryo transfer were performed, which resulted in a clinical pregnancy. The embryo that gave rise to the pregnancy was normal (or balanced) for the inherited CCR, but carried however a trisomy 8 and nullisomy 9 in one of the two biopsied blastomeres. After 8 weeks of pregnancy the couple miscarried. Genetic analysis following embryo-hysteroscopy showed a diploid(90%)/tetraploid(10%) mosaic chorion, while the gestational sac was empty. No aneuploidy 8 was detected in the chorion, while 8% of the cells carried a monosomy for chromosome 9.

We demonstrate that microarray enables to screen against the transmission of the unbalanced meiotic products that can derive from (complex) chromosomal rearrangements. In addition, the findings demonstrate that the genomic constitution of the implanted embryo is not representing the chromosomal rearrangements detected in a single blastomere.

C06.6* Prenatal anomalies in a cohort of 40 Noonan syndrome patients

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Noonan syndrome (NS) is a frequent autosomal dominant disorder caused by mutations in the genes of the RAS/MAPK pathway, being the most frequent PTPN11 and SOS1. Besides the well known post-natal heterogeneous clinical presentation characterized by short stature, congenital heart defects, facial and skeletal dysmorphisms, cryptorchidism, lymphatic dysplasia; the prenatal phenotype is not precisely defined, as well as the related prognostic factors. Several NS anomalies can be observed in fetuses, such as those ones related to lymphatic dysplasia (increased nuchal translucency, cystic hygromas, hydrops fetalis, multiple effusions and polyhydramnios), cardiac defects, and growth retardation. In this retrospective study we evaluated the incidence of abnormal prenatal findings in a cohort of 40 NS patients including 28 PTPN11, 8 SOS1, 1 KRAS, 1 BRAF, 1 RAF1 and 1 SHOC2 mutated patients. Prenatal anomalies were found in 42.5% (17/40) of them : 40% (16/40) polyhydramnios, 17.5 % (7/40) increased fetal nuchal translucency, 17.5 % (7/40) hydrothorax and multiple effusions, 12.5% (5/40) premature rupture of membranes, 2.5 % (1/40) cardiac defects, 9 (22.5%) presenting multiple defects. In conclusion, prenatal anomalies can be found in an elevated fraction of NS patients, being the most common polyhydramnios, with a consistent portion of them presenting multiple anomalies. Interestingly, the observed anomalies do not correlate with the severity of the postnatal phenotype, both at birth and in the first years of life, suggesting that prenatal diagnosis of NS is not burdened by an adverse prognosis.

C07.1 Identification of biomarkers for acute lymphoblastic leukemia (ALL) by allele-specific gene expression and DNA methylation analysis of primary ALL cells

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We used genome-wide allele-specific expression (ASE) patterns to identify genes with *cis*-acting factors that regulate gene expression in acute lymphoblastic leukemia (ALL) cells. We determined the allelic expression levels of 8,000 genes in bone marrow or peripheral blood samples from 197 children with ALL, by quantitative genotyping of single nucleotide polymorphisms (SNPs) in RNA. This analysis identified 391 genes that displayed ASE. Extended promoter regions of these genes were subjected to DNA methylation analysis in cells from 401 patients with childhood ALL. We found that CpG sites located outside "CpG islands" had higher methylation levels and larger variability in CpG site methylation than CpG sites within "CpG islands". We identified 47 genes with an inverse correlation between CpG site methylation and ASE in our ALL sample set, and for 24 genes we observed a correlation between CpG site methylation and ASE levels in individual ALL samples. Using supervised learning, we constructed multivariate classifiers by external cross-validation procedures, and identified genes where the DNA methylation levels allowed accurate discrimination between ALL cells and control cells, between T-lineage and B-cell precursor (BCP) ALL and between the main cytogenetic subtypes of BCP ALL. We also identified 20 genes with DNA methylation levels that predicted relapse of ALL. Most of the genes high-lighted in our study are novel, with no reported connection with ALL. Our findings open up perspectives for more detailed studies on DNA methylation as a

molecular event that leads to ALL and affects the treatment response and clinical outcome.

C07.2* Correlation of telomere length shortening with promoter methylation profile of p16/Rb and p53/p21 pathways in breast cancer

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Unregulated cell growth, a major hallmark of cancer, is coupled with telomere shortening. Measurement of telomere length could provide important information on cell replication and proliferation state in cancer tissues. Telomere shortening and its potential correlation with downregulation of cell-cycle regulatory elements were studied by the examination of relative telomere length and methylation status of the TP53, P21 and P16 promoters in tissues from breast cancer patients. Telomere length was measured in 104 samples (52 tumors and paired adjacent normal breast tissues) by quantitative PCR. Methylation profile of selected genes was analyzed in all samples using a matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Our results demonstrated a significant shortening of tumor telomere regions compared to paired adjacent normal tissues ($P<0.001$). Similarly, telomere lengths were significantly shorter in advanced stage cases and in those with higher histological grades ($P<0.05$). Telomere shortening in cancer tissues was correlated with a different level of hypermethylation in the TP53, P21 and P16 promoters ($r=-0.33$, $P=0.001$; $r=-0.70$, $P<0.0001$ and $r=-0.71$, $P<0.0001$, respectively). The results suggested that inactivation of p16/Rb and/or p53/p21 pathways by hypermethylation may be linked to critical telomere shortening, leading to genome instability and ultimately to malignant transformation. Thus, telomere shortening and promoter hypermethylation of related genes both might serve as breast cancer biomarkers.

C07.3* Discovery of molecular targets for therapy by expression profiling of thyroid cancer cases

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Thyroid cancer represents a heterogeneous entity and its clinical characteristics vary widely. Gene-expression profiling studies have identified a variety of potential molecular markers to help distinguish benign from malignant thyroid neoplasms.

To elucidate the mechanisms underlying the progression and to identify novel therapeutic targets, we assessed the genome-wide expression in normal and tumor thyroid tissues.

Pure populations of malignant and healthy cells were isolated by applying the technology of LCM. We compared the gene expression patterns of 18 malignant thyroid samples and 13 samples of normal epithelial non-transformed cells using the GeneChip trademark from Affymetrix.

We identified 243 probes that were significantly differentially over-expressed and whose ratio tumor/control was more than 5 in more than 50% of the analyzed samples. Among the top overexpressed genes in thyroid carcinoma were: *FN*, *CHI3L1*, *FAM20A*, *SEACAM6*, *NMU*, *LPL* etc. Unsupervised hierarchical clustering separated papillary from medullar carcinoma samples. The differential expression of 7 genes was validated by quantitative real-time PCR. Two of those candidates (*RGS4* and *QPCT*) were confirmed by immunohistochemistry, Northern blot analyses and further functional analysis, including siRNA experiments. Based on this result, we consider *RGS4* as a good molecular target for therapy of thyroid cancer. Thus, the molecular signatures unique to thyroid carcinoma provide a molecular basis for therapeutic target discovery.

C07.4* A new approach for ovarian cancer screening - characterization of miRNA profiles in peripheral blood

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Screening is an unsolved problem for ovarian cancer, especially as late detection is equivalent to poor prognosis. Thus, we investigated whether ovarian cancer patients display characteristically deregulated miRNAs in blood cells that could form the basis of a test for disease progression or even for preventive screening. To this aim, blood-borne miRNA profiles from 15 patients with ovarian cancer and 15 age- and sex-matched healthy controls were compared and biostatistically evaluated. This showed that 51 out of >900 tested miRNAs were deregulated by unadjusted Student's t-test. While some candidates had already been linked with cancer (e.g. miR-155), most had never been connected to a specific disease before. Bioinformatics further enabled us to define a miRNA profile which allowed for discrimination between blood samples of ovarian cancer patients and healthy controls with an accuracy and specificity of >70%. When only cancers of the serous subtype were considered and compared with an extended control group ($n=37$), sensitivity exceeded 90%.

Taken together, our proof-of-principle study strengthens the hypothesis that neoplastic diseases generate characteristic miRNA fingerprints in blood cells. Thus, we propose that the combination of microarray-based miRNA-profiling from peripheral blood with other markers might improve the notoriously difficult but important screening for ovarian cancer.

C07.5* Methylation Profiles of 22 Candidate Genes in Breast Cancer Using High-Throughput MALDI-TOF Mass Array

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Alterations of DNA methylation patterns have been suggested as biomarkers for diagnostics and therapy of cancers. Every novel discovery in the epigenetic landscape and every development of an improved approach for accurate analysis of the events may offer new opportunity for the management of patients. Using a novel high-throughput mass spectrometry on MALDI-TOF silico-chips, we determined quantitative methylation changes of 22 candidate genes in breast cancer tissues. For the first time we analysed the methylation status of a total of 42,528 CpG dinucleotides on 22 genes in 96 different paraffin-embedded tissues (48 breast cancerous tissues and 48 paired normal tissues). A two-way hierarchical cluster analysis was used to classify methylation profiles. In this study, 10 hypermethylated genes (*APC*, *BIN1*, *BMP6*, *BRCA1*, *CST6*, *ESRb*, *GSTP1*, *P16*, *P21* and *TIMP3*) were identified to distinguish between cancerous and normal tissues according to the extent of methylation. Individual assessment of the methylation status for each CpG dinucleotide indicated that cytosine hypermethylation in the cancerous tissue samples was mostly located near the consensus sequences of the transcription factor binding sites. These hypermethylated genes may serve as biomarkers for clinical molecular diagnosis and targeted treatments of patients with breast cancer.

C07.6 Unraveling the Complexity of Primary and Metastatic Ewing's Sarcoma Using Helicos Single Molecule Sequencing

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Ewing sarcoma is a high-risk childhood cancer that represents a major treatment challenge, as survival has not improved significantly despite aggressive chemotherapy. Elucidation of molecular changes in progression of this cancer towards the metastatic and chemo-resistant states offers the potential to better understand the fundamentals of biological processes responsible for poor disease outcome. We have applied Helicos single-molecule sequencing to build unbiased whole-genome profiles of the transcriptome, methylome and DNA of two cell lines derived from the primary tumor of a Ewing sarcoma patient and her chemo-resistant metastasis. This simple system is ideal for generation of hypotheses on relationships between coding and non-coding RNAs, the epigenome, primary DNA sequence, and the malignant phenotype. Unlike work on primary tumors, it also allows for biologic validation.

Large differences between the primary and metastatic cells exist at both the genomic and transcriptome levels, including differential expression and methylation as well as copy number and sequence variants suggesting clonal evolution from the primary to the metastatic tumor. A significant fraction of the genome was found to represent RNAs expressed at different levels between the cell lines, of which ~60% corresponded to un-annotated transcripts. In some instances, the latter could span large (100's of kb) genomic regions that contain no annotated transcripts. About 25% of the transcripts upregulated in the metastatic clone were found to be associated with promoter demethylation when compared to the primary tumor, suggesting that promoter demethylation is specifically associated with up-regulation of transcripts in the metastatic/chemo-resistant tumor in a selective manner.

C08.1 Treacher Collins syndrome - detailed genetic and phenotypic analysis

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The Treacher Collins syndrome (TCS, OMIM #154500) is an autosomal dominant condition characterised by craniofacial dysmorphism consisting of downslanting palpebral fissures, lower eyelid coloboma, hypoplasia of facial bones and microtia. Mutation analysis of the *TCOF1* gene identifies pathogenic mutations in more than 90% of patients with typical TCS. In 226 patients referred to us as having TCS, we performed *TCOF1* sequence and MLPA (26/28 exons) analyses. Point mutations scattered throughout the gene were identified in 78 of them, but no deletion or duplication was found in the remaining 148. By careful evaluation of all available clinical data of these 148 patients, we could confirm the suspected diagnosis of TCS in only 10 of them. These were then subjected to SNP-array analyses, but no deletion or duplication was found.

We will report in detail on the clinical findings in 49 index patients, including 21 new ones, and their affected family members with a pathogenic mutation in the *TCOF1* gene and will show average 2D faces we created from standardized photographs. We observed a wide inter- and intrafamilial phenotypic variability. Possibly stochastic variation or unidentified genetic modifiers, most likely not in *cis* might contribute to the phenotypic variability.

Some members of families with molecularly proven TCS are so mildly affected that they do not even fulfill the minimal diagnostic criteria defined earlier (Teber et al., 2004). Thus, a genotype phenotype correlation can not be made. The widened phenotypic spectrum must be considered in genetic counselling.

C08.2 Genomic rearrangements of the *GREM1-FMN1* locus cause Oligosyndactyly, Radio-Ulnar synostosis, Hearing loss, Renal defects syndrome and Cenani-Lenz-like non-syndromic Oligosyndactyly

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Limb development is a complex process requiring proper spatio-temporal expression of a network of limb specific morphogens. *Grem1* and

Fmn1 play an important role in mouse and chick limb development. The mouse *limb deformity (ld)* phenotype with digit reduction, syndactyly, radio-ulnar synostosis, variable renal defects and absent fibulae is caused by loss of *Grem1* function. This could be due to either coding *Grem1* homozygous mutations or homozygous deletions of the neighbouring *Fmn1* gene, which also removes limb specific regulatory sequences of *Grem1*. Recent studies reinforce the hypothesis that a loss of *Fmn1* protein could also contribute to the observed *ld* anomalies. In addition, an over-expression of *Grem1* in developing chick limbs represses the programmed cell death in the interdigital mesenchyme, resulting in interdigital webbing and truncation of distal cartilage elements.

We report here, for the first time, chromosomal imbalances in the *GREM1-FMN1* region in individuals with limb defects. A 263Kb homozygous deletion of *FMN1* was associated with oligosyndactyly, radioulnar synostosis, hearing loss and renal defects, features identical to *ld* mice. A 1.7Mb duplication encompassing both the *GREM1* and *FMN1* genes was detected in an isolated Cenani-Lenz-like oligosyndactyly of the hands, resembling the transgenic chick wings in which *Grem1* was over-expressed. The phenotypes of the patients represent new entities/syndromes within the Cenani-Lenz clinical spectrum- (1) an autosomal recessive Oligosyndactyly, Radio-Ulnar Synostosis, Hearing loss and Renal defect syndrome and (2) an autosomal dominant Cenani-Lenz-like non-syndromic Oligosyndactyly.

C08.3 An inherited arthritis is caused by mutations in *TRPV4*

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Familial Digital Arthropathy-Brachydactyly (FDAB; OMIM 606835) is a dominantly inherited condition involving aggressive osteoarthritis of the fingers and toes and consequent shortening of the middle and distal phalanges. We show, in two unrelated families, that the disorder is caused by missense substitutions (G270V, R271P and F273L) in the intracellular ankyrin repeat domain of the transient receptor potential cation channel *TRPV4*. Functional testing of mutant *TRPV4* in stably transfected HEK293 cells revealed the mutant proteins showed poor cell surface localization, despite being expressed at similar levels to wild type protein, and calcium influx in response to the *TRPV4* agonist GSK1016790A was significantly reduced. Others have recently shown that gain of function *TRPV4* mutations cause a range of skeletal dysplasias and peripheral neuropathies. Our data, showing tightly clustered *TRPV4* mutations that reduce channel activity in a third phenotype, inherited osteoarthritis, demonstrate the importance of *TRPV4* activity in articular cartilage homeostasis and raises the possibility that this cation channel might play a role in age-related osteoarthritis.

C08.4* Mutations in *PITX1* cause a human patellar malformation syndrome: Delineation of a recognisable lower-limb phenotype

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The identification of gene defects in human patellar malformation syndromes and mutant animal models proved to be important in unravelling key molecules involved in patellar development and musculoskeletal patterning. The nail patella syndrome phenotype emphasised that the LIM homeodomain transcription factor LMX1B is critical in dorsoventral and anteroposterior patterning of the upper- and lower-limbs, as well as the formation of patellar/lilac/radial bones. Phenotype studies of the small patella syndrome (SPS) determined that the T-box transcription factor TBX4 is implicated in dorsoventral and anteroposterior patterning of the lower-limb and late skeletal development of patellar/ischiopubic/(meta)tarsal bones. We aimed at elucidating the

molecular cause in four classical SPS families and six families with a SPS-like phenotype, in which sequencing of *TBX4*, *TBX2*, and *LMX1B* was found normal. The paired-like homeodomain transcription factor *PITX1* was selected as a strong candidate gene for congenital patellar, pelvic and foot malformations, based on its role as an upregulator of *Tbx4* in the determination of lower-limb identity in chicken and mice. *Pitx1* knockout mice showed absent patellae and lower-limb bones resembling an upper limb-like morphology. We identified two different putative loss-of-function mutations (one missense and one nonsense) in *PITX1* in one four-generation and one two-generation SPS-like family, respectively. The phenotypic spectrum comprised aberrant patellar size and morphology, unilateral clubfoot and iliac/tibial/fibular/talus/calcanal bone malformations. The present identification of *PITX1* mutations in man, together with the phenotype of animals lacking *Pitx1* delineates a recognisable autosomal dominant patellar malformation syndrome resulting from disrupted anteroposterior patterning and left-right directional asymmetry of the lower-limb.

C08.5* TRPV4-related skeletal dysplasias: a clinical, radiographic, and molecular study in 18 families.

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The transient receptor potential vanilloid 4 protein (TRPV4) is a calcium-permeable ion channel that responds to many different stimuli, is widely expressed, and participates in an extraordinarily wide range of physiologic processes.

Autosomal dominant brachyolmia, spondylometaphyseal dysplasia Kozlowski type (SMDK) and metatropic dysplasia are three distinct skeletal dysplasias which share some common features, including short stature, platyspondyly, and progressive scoliosis. SMDK and metatropic dysplasia also have significant but variable metaphyseal involvement on radiographs.

In the past two years, mutations in the gene encoding TRPV4 have been found to be responsible for these three skeletal phenotypes, confirming that they are allelic disorders and suggesting that they might represent different parts of a phenotypic spectrum.

We analysed the clinical, radiographic and molecular data on 21 patients from 18 families, all of whom had a clinical diagnosis of one of the conditions described above: 12 with metatropic dysplasia; 3 with SMDK; and 3 with brachyolmia. Our study identified 7 different mutations in 14 out of the 18 studied families, two previously described, and 5 novel.

These data have uncovered new genotype-phenotype correlations and suggest that these three conditions represent a continuum of severity with areas of phenotypic overlap between conditions, even within the same family. We hope that these data will also add to the understanding of the molecular basis of these disorders and identify possible pharmacologic targets for therapy.

C08.6 Clinical and molecular findings on 20 patients with fibrodysplasia ossificans progressiva

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Fibrodysplasia ossificans progressiva (FOP) (MIM 135100) is a rare genetic disorder of progressive extraskeletal ossifications. The heterozygous mutation c.617G>A (p.R206H) in the ACVR1 gene is regarded as the cause of FOP in classically affected individuals worldwide (Shore et al. 2006).

We report on clinical findings of 20 patients (10 female, 10 male pa-

tients, aged eight months to 38 years) with FOP and on molecular findings of 13 of them. Most of them show a typical hypoplasia/aplasia and valgus deviation of the great toe (17/20). A common symptom are painful swellings on shoulders and face beginning to arise approximately at an age of three years (15/20). Hand anomalies, such as thumb hypoplasia (18/20) and clinodactyly of the fifth finger (15/20) are common. Some patients have hypoacusis. Further features are restricted mobility of the spine and contractures of other joints as a result of heterotopic ossifications. Some patients show craniofacial features like hypomimia, sparse eyebrows and hair and teeth anomalies.

Sequence analysis of the ACVR1 gene were performed on 13/20 patients. Ten patients show the typical mutation p.R206H. Two patients with an atypical phenotype show different mutations (G328W and G356D).

We analysed the metacarpophalangeal profile (MCP) on X-rays of seven patients. We suggest that there is a typical MCP pattern in classical FOP, which could be used as an additional diagnostic tool. We discuss the clinical, radiological and molecular findings of our patients and compare them with the literature. Our study contributes to the understanding of the FOP phenotype and possible genotype-phenotype correlations.

C09.1 Chromosome-wide mapping of long-range interactions involved in Smith-Magenis and Potocki-Lupski syndromes

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Copy number variations (CNVs) affect expression levels of the genes that map within the affected region, but also of genes located in the flanking regions. To understand the mechanisms at play, we studied gene expression and chromatin conformation in mice models of Smith-Magenis (SMS) and Potocki-Lupski (PTLS) syndromes, containing a microdeletion and its reciprocal microduplication, respectively, on mouse chromosome 11 (MMU11). We profiled the transcriptome of embryonic fibroblasts of mice with one, two, three and uniallelic two copies of the SMS/PTLS region in an otherwise identical genetic background. As expected, the most differentially expressed transcripts are mapping to the SMS/PTLS interval, but a significant proportion of most differentially expressed genes also map to the rest of MMU11. We hypothesized that these chromosome-wide effects might be caused by changes in long-range interactions along the entire chromosome. We therefore analyzed the chromatin structure of these four mouse strains by using the chromosome conformation capture carbon copy (5C) technology. This will allow comparing the chromatin structure and the presence of physical contacts between functionally interacting genomic elements in genotypes differing only by the number of copies of a CNV, and correlating these interaction maps with the observed differential gene expression. Detailed investigations of the different mechanisms by which CNVs alter the architecture of chromosomes are warranted to shed light on how CNVs influence gene expression. In addition, this study will provide a first comprehensive chromatin interaction map of an entire mouse chromosome.

C09.2* Kidney-specific inactivation of Ofd1 leads to renal cystic disease associated to upregulation of the mTOR pathway

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The Oral-Facial-Digital type I syndrome (OFDI; MIM 311200) is a rare syndromic form of inherited renal cystic disease. It is transmitted as an X-linked dominant, male lethal disorder and is caused by mutations in the OFD1 gene. Previous studies demonstrated that OFDI belongs to the growing number of disorders ascribed to dysfunction of primary cilia. We generated a conditional inactivation of the mouse Ofd1 gene using the Ksp-cre transgenic line, which resulted in a viable model characterized by renal cystic disease and progressive impairment of renal function. The study of this model allowed us to demonstrate that

primary cilia initially form and then disappear after the development of cysts, suggesting that the dysfunction of primary cilia is a consequence rather than the primary cause of renal cystic disease. Immunofluorescence and western blotting analysis revealed upregulation of the mTOR pathway in both dilated and non dilated renal structures. Treatment with rapamycin, a specific inhibitor of the mTOR pathway, resulted in a significant reduction in the number and size of renal cysts and a decrease in the cystic index compared with untreated mutant animals, suggesting that dysregulation of this pathway in our model is mTOR-dependent. The animal model we have generated could thus represent a valuable tool to understand the molecular link between mTOR and cyst development, and eventually to the identification of novel drug targets for renal cystic disease.

C09.3 Lack of *Mid1*, the mouse ortholog of the Opitz Syndrome gene, causes abnormal development of the anterior cerebellar vermis

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Opitz G/BBB Syndrome (OS) is a genetic disorder characterized by midline developmental defects. Male patients with the X-linked form of OS, caused by loss-of-function mutations in the *MID1* gene, show high variability of the clinical signs. *MID1* encodes a ubiquitin ligase that controls Phosphatase 2A but its role in the pathogenesis of the disease is still unclear. Here we report a mouse line carrying a non-functional ortholog of the human *MID1* gene, *Mid1*. *Mid1* null mice show the brain anatomical defect observed in patients, i.e. hypoplasia of the anterior portion of the medial cerebellum, the vermis. We found that the presence of this defect correlates with motor coordination, procedural and non-associative learning impairments. The defect is limited to the most anterior lobes of the vermis, the region of the developing cerebellum adjacent to the dorsal midbrain. Analyses at mid-gestation reveal that lack of *Mid1* causes the shortening of the posterior dorsal midbrain; the rostralization of the midbrain/cerebellum boundary; and the down-regulation of a key player in the development of this region, *Fgf17*. Thus, lack of *Mid1* causes a mis-specification of the midbrain/cerebellar boundary that results in an abnormal development of the most anterior cerebellar lobes. This animal model provides a tool for further *in vivo* studies of the physiological and pathological role of the *Mid1* gene and a system to investigate the development and function of anterior cerebellar domains.

C09.4* Global gene transfer in the CNS and phenotypic correction of MLD model mice by systemic neonatal injection of serotype 9 AAV vector

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Lysosomal storage diseases (LSDs) are important targets for enzyme replacement and gene therapy. The success of gene therapy for LSDs with neurological involvement such as metachromatic leukodystrophy (MLD) depends on the development of efficient delivery of lysosomal enzymes and/or vectors across the blood-brain barrier (BBB) to achieve wide distribution of enzyme throughout the brain. Since both the immune system and the BBB are developmentally immature during the perinatal period, neonatal gene transfer may be a highly promising strategy to treat genetic neurological disorders. To treat MLD mice, we generated serotype 9 AAV vector expressing human arylsulfatase A (AAV9/ASA) and IV injected into neonatal MLD mice (n=7). ELISA analysis showed that sustained expression of ASA was detected in the brain (cerebral cortex: 24.5±8.7, cerebellum: 7.8±4.5 ng/mg protein) for more than one year. Alcian blue staining and quantitative analysis of sulfatide contents by biochemical assay showed significant decrease of the amount of stored sulfatide in AAV9/ASA treated MLD mice compared to non-treated mice (cerebral cortex: 12.0±5.3 vs. 29.7±12.7, p<0.03; cerebellum: 34.8±6.3 vs. 73.2±5.0, mg/mg protein, p<0.05). Furthermore, in the behavior test, AAV9/ASA treated mice showed a significant improvement in their ability to traverse narrow balance beams, as compared to non-treated MLD mice (Latency: 9.2±1.5 vs. 13.0±0.4 sec, P<0.05; Slips: 3.5±1.9 vs. 4.3±1.4 times, P<0.05). These data clearly demonstrate that MLD model mice can be treated by systemic neonatal injection of AAV9/ASA. Therefore, neonatal gene

therapy may be an important option for parents faced with the prenatal diagnosis of a genetically affected child.

C09.5* The forkhead transcription factor FOXL2 is sumoylated in both human and mouse: Sumoylation affects its stability, localization, and activity

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The FOXL2 forkhead transcription factor is expressed in ovarian granulosa cells, and when mutated causes the Blepharophimosis, Ptosis and Epicanthus Inversus Syndrome (BPES) and predisposes to premature ovarian failure. Inactivation of Foxl2 in mice demonstrated its indispensability for female gonadal sex determination and ovary development and revealed its antagonism of Sox9, the effector of male testis development.

To define FOXL2 regulatory activities we looked for interacting proteins. Based on yeast two-hybrid screening, we found that FOXL2 interacts with PIAS1 and UBC9, both parts of the sumoylation machinery. We confirmed the interactions by co-immunoprecipitation and we demonstrated that human FOXL2 is sumoylated in transfected cell lines, and that endogenous mouse Foxl2 is comparably sumoylated. Confocal microscopy allowed us to demonstrate that FOXL2 co-localizes with SUMO-1 in structures resembling PML (promyelocytic leukaemia) bodies in transfected cells and that FOXL2, SUMO1 and UBC9 co-localize *in vivo* in 4 weeks old mouse ovary. We identified 7 putative sumoylation sites using Abgent SUMOPlot™ software, and created FOXL2 mutants in which the lysines of the higher score putative sumoylation sites (K25, K87, K114, K150) were changed to arginine. Our results indicate that K114 and K150 are involved in FOXL2 sumoylation and nuclear localization and that all mutations influenced FOXL2 transcriptional activity. Furthermore we demonstrated that sumoylation results in an increase of FOXL2 protein levels, likely due to an increase in protein stability. It is intriguing that similar sumoylation and regulatory consequences have also been reported for SOX9, the male counterpart of FOXL2 in somatic gonadal tissues.

C09.6 SAHA ameliorates the SMA phenotype in two mouse models for spinal muscular atrophy

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Proximal spinal muscular atrophy (SMA) is a common autosomal recessively inherited neuromuscular disorder determined by functional impairment of α-motor neurons within the spinal cord. SMA is caused by functional loss of the *survival motor neuron gene 1* (*SMN1*), whereas disease severity is mainly influenced by the number of *SMN2* copies. *SMN2*, which produces only low levels of full-length mRNA/protein, can be modulated by small molecules and drugs, thus offering a unique possibility for SMA therapy.

Here, we analysed suberoylanilide hydroxamic acid (SAHA), a FDA-approved histone deacetylase inhibitor, as potential drug in two severe SMA mouse models each carrying two *SMN2* transgenes: US-SMA mice with one *SMN2* per allele (*Smn*^{-/-}; *SMN2*^{tg/tg}) and Taiwanese-SMA mice with two *SMN2* per allele (*Smn*^{-/-}; *SMN2*^{tg/wt}), both on pure FVB/N background. The US-SMA mice were embryonically lethal with heterozygous males showing significantly reduced fertility. SAHA-treatment of pregnant mothers rescued the embryonic lethality giving rise to SMA offspring. By using a novel breeding strategy for the Taiwanese model (*Smn*^{-/-}; *SMN2*^{tg/tg} x *Smn*^{-/-} mice) we obtained 50% SMA offspring that survive ~10 days and 50% control carriers in each litter. Treatment with 25 mg/kg/2x/day SAHA increased lifespan of SMA mice by 30%, significantly improved motor function abilities, reduced degeneration of motor neurons within the spinal cord and increased the size of neuromuscular junctions and muscle fibers compared to vehicle-treated SMA mice. *SMN* RNA and protein levels were significantly elevated in various tissues including spinal cord and muscle. Hence, SAHA, which lessens the progression of SMA, might be suitable for SMA therapy.

C10.1 Copy number variable regions in 13 European populations

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At present time there is no comprehensive copy number variable region (CNVR) map of European population although it is a prerequisite for the European-wide collaborative GWA studies. Most of the efforts so far have been directed into the characterization of the fine-scale CNVR structure in HapMap but Europe as a whole, especially the Eastern part, has not been covered in sufficient detail.

We have studied about 2,000 individuals from thirteen cohorts from Europe (including about 900 Estonians) and focused on describing the genetic structure, especially the copy number variable regions (CNVR), in Eastern Europe and in Europe as whole.

In total we identified more than 2,500 CNVR in 1,080 individuals (including only 100 randomly chosen Estonians). When analyzing only the Estonians, we identified more than 5,010 CNVR (maf cut-off 1%) and the majority of CNVR's had a very low frequency in the population. For the identified regions, principal component (PC) analysis were applied and the resulting map was not as informative geographically as in the case of SNP data. The CNVRs included in the 20 first PC show an important contribution from the HLA region, and the Immunoglobulin variable regions, while GO pathway analysis identifies a cluster of genes for sensory perception of chemical stimulus.

In addition to SNP's, CNV's are being used as additional genetic markers in GWAS studies for disease gene mapping. The results presented here add new data to European genetic map of the structural variations that will greatly facilitate inter-population genetic studies.

C10.2 A founder mutation in LEPRE1 causes lethal recessive Type VIII Osteogenesis Imperfecta and occurs in West Africans and African Americans

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Recessive osteogenesis imperfecta (OI) is caused by defects in cartilage-associated protein (CRTAP) or prolyl 3-hydroxylase 1 (P3H1/LEPRE1), which form the collagen prolyl 3-hydroxylation complex with cyclophilin B (PPIB). Deficiency of P3H1 causes severe to lethal

type VIII OI (OMIM #610915). We have identified a LEPRE1 mutation, IVS5+1G>T, in unrelated African Americans (AA) and contemporary West Africans (WA) immigrants. Screening of gDNA from a Mid-Atlantic AA cohort determined a carrier incidence of 0.30-0.50% for this mutation, predicting occurrence of homozygous lethal type VIII OI in about 1/250,000 births in this population. To trace the mutation origin, we screened gDNA from a contemporary WA cohort. Nineteen of 1284 unrelated individuals (1.48%) from Nigeria and Ghana were heterozygous carriers, half of whom were from the Yoruba or Ibo ethnic groups of Nigeria. The high carrier frequency for this founder mutation among WAs predicts that this mutation alone would cause recessive OI in 1/18,250 births in WAs, equal to the incidence of de novo dominant OI in North America. To estimate the mutation age, we examined microsatellites and short tandem repeats spanning 4.2 MB surrounding the LEPRE1 gene. Disease allele haplotypes were determined from 12 contemporary WA and 3 AA families. WA carriers share a haplotype of 175-425Kb. Using the linkage disequilibrium analysis method of Rannala & Slatkin (2000), the mutation was estimated to have originated 800-960 years ago (1050-1210 C.E.). This timing is consistent with the model that this West African allele was transported to the Americas via the Atlantic slave trade (1450-1860 C.E.).

C10.3 Paleogenetic study for reconstruction of genealogy of first Moldavian princes from 14th century

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We performed paleomolecular genetic analysis on skeletal remains of 7 princes of Moldavia from the last half of the 14th century, buried in the „Saint Nicholas“ Church from Radauti-Romania, in order to reconstruct their genealogy. We used the Amelogenin gene for identifying the genetic sex of the old individuals and we analysed mitochondrial (HVR I region) and nuclear DNA (VWA31A, TPOX, DYS392 and DYS393 Microsatellites) polymorphisms to reveal their genetic kinship along both maternal and paternal lineages. Ancient DNA was extracted by a phenol-chloroform-based method and amplified by PCR. The HVR I mitochondrial DNA sequences and nuclear DYS392 and DYS393 polymorphic markers were sequenced, and the other nuclear DNA markers were separated and identified on Polyacrylamide gels (Ag-stained). Our results revealed the genetic kinship between the 7 Moldavian princes, showing two male lines with a maternal linkage between the two groups. The first male line consisted of two individuals close related to each other and differed from the other 5 individuals also closed related among them as concerns the paternal line. This evidence argued that two princely dynasties succeeded on the Moldavian sceptre in the last half of the 14th century, contrary to the historiography version which had considered the existence of only one dynasty and succession exclusively along the paternal line.

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C10.4* Modelling haplotype structure in isolate populations for population sequencing

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Isolated founder populations are a powerful tool for disease genetics. By sequencing a modest number of individuals, we should be able to identify most founding haplotypes present in current individuals, then use long-range phasing and imputing to derive accurate and near complete genome sequence for all genome wide genotyped individuals within the population.

To design such a study, we have developed a method to fit population history simulations to the observed pairwise IBS segment length distribution from dense genotype data. Using this approach with 7505 markers on chromosome 20, we created a genetic model for Kuusamo, a well known isolate population in North Eastern Finland. According to historical records, Kuusamo had small indigenous Lapp population until its „resettlement“ by 34 families, totalling 136 chromosomes, in the 1680s. The current census population size is 25108, including some recent immigrants.

Our best fit model has a population of 100 founding individuals ex-

panding first rapidly, then more slowly, to a current effective population size of 7930 in 12 generations, rejecting significant historic immigration after the initial resettlement. The simulation suggests that out of the 200 founding haplotypes only 108 ± 10 survive today per locus, the rest having been lost by genetic drift. On average, 80% of a present day chromosome is inherited from the founders in fragments of length 6cM or more. The results suggest that a sample of 200 individuals would cover 93-98% of current indigenous chromosome population. We are exploring using this approach to model other European isolates.

C10.5* Inverse mapping approach implies the role of large CNVs in intellectual deficits and learning difficulties in a population cohort

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The availability of genome wide data on representative population samples provides an opportunity to apply a strategy of inverse mapping for correlating human traits with genotypes. Whereas the traditional forward mapping aims to define genotypic sharing accounting for a common phenotype among group of individuals, the inverse mapping seeks to discover phenotypic features shared among individuals demonstrating allelic similarity. This approach would avoid the inherent imprecision in phenotype definition that makes it difficult to determine a priori which individuals are sharing a phenotype. Here we provide a proof of principle of this inverse mapping approach by systematically scanning all CNVs >500 kb in a population cohort (N= 4,932). The participants were drawn from a prospective birth cohort of all individuals born in 1966 in North of Finland. Routine follow-ups have resulted in extensive phenotype database collected from the study participants. We identified 634 large CNVs observed in 529 individuals. To narrow down our data inquiries to a reasonable number of phenotypes, we focused on a category of traits postulated to relate with previous CNV findings in neuropsychiatric deficits. We observed significantly higher frequencies of cognitive defects defined as 8 among carriers of large deletions (5.0%) compared to non carriers (1.4%) ($p < 0.0024$). Intriguingly, the deletion carriers were also more likely to present with sub clinical learning difficulties than the general population (10% vs. 3.9%; $p=0.00088$). The study highlights the opportunity to utilize inverse mapping as a strategy to characterize phenotypic consequences related to genetic variants in an unbiased population sample.

C10.6* A novel approach to analysis of raw Illumina microarray data to assess the contribution of copy number variations to obesity and gene expression

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Introduction:

Obesity is a major contributor to ill health worldwide. Exploration of the genetic contribution to obesity using genome wide approaches has revealed both SNP and CNV associations. Here, we have used a novel approach to analysis of microarray-derived GWAS data to assess the contribution of CNVs to obesity and gene expression.

Subjects and Methods:

The SOS SibPair cohort consists of 154 nuclear families (732 individuals), each containing a body mass index-discordant sib pair (≥ 10

kg/m² difference). Gene expression and genotyping data was generated from 349 siblings using the Affymetrix Human U133 Plus 2.0 and Illumina Infinium Human 610K Quad platforms, respectively. RNA was extracted from subcutaneous adipose tissue and DNA was extracted from blood. Analysis was performed modelling genotyping signal intensity levels (LogR Ratio and B Allele Frequency) and gene expression levels in a variance component framework.

Results:

We detected more than 10 genomic regions where signal intensity was significantly associated with expression levels. For example, in a region on chromosome 6 overlapping the MHC, we identified 34 markers associated with transcript expression level at genome wide significance ($p \leq 10^{-7}$). As expected, these regions are listed in the Database of Genomic Variants, i.e. they are CNVs.

Conclusions:

Using quantitative analysis of raw Illumina microarray data, we have identified more than 10 genomic regions that are associated with expression levels ($p \leq 10^{-7}$). We will investigate these further, comparing them to the genes that are differentially expressed between obese and lean subjects, to identify candidate genes for obesity.

C11.1 Policy Recommendations of the PPPC on direct-to-consumer genetic testing for health purposes

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An increasing number of private companies are now offering direct-to-consumer (DTC) genetic testing services, ranging from tests for single gene, highly penetrant disorders to susceptibility testing for genetic variants associated with common complex diseases or with specific traits. The Professional and Public Policy Committee of the European Society of Human Genetics is concerned about the way commercial companies are currently introducing new genetic tests into the market and outside of the scope of the traditional health care system. Therefore, it is currently in the process of drafting policy recommendation on the topic of direct-to-consumer sales and/or advertising of genetic tests for health purposes. This presentation wants to draw the attention to this document and intensify the debate on this topic. The presentation will discuss following topics: the right to information, the advertising of genetic tests, the quality of genetic testing services (the quality of the genetic tests -in terms of validity and utility-, the quality of laboratories and the quality of the persons providing the genetic services), the individualized medical supervision, information and genetic counseling, informed consent, genetic testing of minors, respect for private life, research, oversight of genetic testing, and the impact on the health-care system.

C11.2* Reporting genetic research results: A quasi-experimental approach to understanding researchers' judgments

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Background: Ethicists suggest that researchers are obliged to report individual genetic research findings to study participants. Opponents contend that promising or reporting provisional knowledge may cause harm and that clinical follow-up regarding these findings may not be available to research participants. Methods: A cross-sectional quasi-experimental survey using vignettes to understand factors that influence researchers' judgments about this obligation was administered to cystic fibrosis and autism researchers, internationally. Results: 80% of 342 researchers agreed that individuals in whom a genetic variation is identified should be informed of this finding if judged to be clinically significant. Researchers felt 30-70% less confident that a given finding was clinically significant when it was related to autism research, less scientifically robust, incidental to the index condition, and when barriers to receiving clinical services were perceived ($p < .05$). Researchers were 30% less likely to report scientifically weaker findings and 40% less likely to report findings when they lacked capacity to provide requisite medical care ($p < .05$). Compared to molecular and statistical researchers, clinical researchers were 1.8 times more likely to endorse the significance of a given finding and 1.5 times more likely to report it to participants ($p < .05$). Conclusion: Judgments about reporting genetic research results are influenced not only by scientific parameters, but also by the role of the researcher and his/her capacity to meet par-

participants' clinical needs when provisional knowledge is reported. Given the complexity reflected herein, we question the appropriateness of an unequivocal imperative to disclose individual research findings to study participants.

C11.3 Direct-to-consumer genetic testing companies: what are their policies regarding testing in minors?

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Advances in genetic knowledge and technologies have increased the possibilities of testing asymptomatic minors for late-onset diseases, carrier status and susceptibility to common complex disorders. In addition, there has been a recent increase in companies offering genetic tests directly-to-consumers and bypassing the traditional face-to-face encounter with a health care professional from the established health-care system. With this in mind, we gathered information regarding policies on testing in minors from the websites of 29 companies offering direct-to-consumer (DTC) health-related genetic testing. The results of this study showed that almost half of the companies did not present any information regarding a policy for testing in minors, and that many of the remaining companies had ambiguous statements regarding this issue. Therefore, as a follow up to this content analysis, we sent surveys to 37 companies (including the 29 companies that were part of our initial study) asking them specific questions about their policy on genetic testing in minors. The analysis of survey responses is underway and will provide information not only about companies' policies, but also about the actual demand for testing in this population. Given the concerns about the ethical, legal and psychological implications of performing genetic tests in healthy children, it is important to monitor the activities of DTC genetic testing companies in this regard.

C11.4* Genetic Counsellors' Views Regarding Their Role in Delivering a Pharmacogenetic Service

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Currently, there is no clear resolution on which healthcare profession should play a role in delivering a pharmacogenetic service. In addition, there is limited published evidence on healthcare professionals' views on pharmacogenetic services. This study aimed to explore the views of genetic counsellors on pharmacogenetic services. Semi-structured face-to-face interviews were carried out with 14 genetic counsellors from two regional genetics clinics in England. The interviews comprised open questions designed to explore their views on potential (dis)advantages of pharmacogenetic testing and the role of counsellors and other professionals in delivering a pharmacogenetic service. In addition, four vignettes describing types of pharmacogenetic tests were used to elicit the counsellors' views on specific clinical applications. Data were analysed using the constant comparative method to identify key themes. Opinions on whether genetic counsellors should be involved in a pharmacogenetic service were varied. The genetic counsellors interviewed did see a potential role for their profession but further training may be needed due to their limited knowledge of pharmacogenetics and drug prescription. A regional genetics centre was perceived to be an inappropriate clinical setting for a pharmacogenetic service. However, employing genetic counsellors in a specialist role within a multidisciplinary clinic could be beneficial. Genetic counsellors' responses to the vignettes suggested that different pharmacogenetic tests are likely to have different issues, in terms of setting up models of service delivery, and implications for patients. Therefore any clinical service model developed for pharmacogenetic testing will need to be adapted according to the specific issues each test presents.

C11.5 Informed consent for large-scale biobank research: experience and attitudes of cancer patients

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Background: The transfer of biological samples between biobanks is developed to foster the development of large-scale multicentric projects. However, the "commercial" use of samples and the indispensable concomitant transfer of clinical data associated might be a concern for

cancer patients.

Objectives: We assessed patients' experience and attitudes towards the biobank informed consent process.

Methods: A mixed-methods design was used. Nineteen patients (aged 28-82) were in-depth interviewed using grounded theory methodology and 574 patients (aged 20-89, response rate=77.0%) treated for colorectal, breast cancer or a haematological malignancy answered to a self-administered questionnaire.

Findings: Two hundred thirteen patients (37.1%) declared they had given consent. Among them less than the half (41.7%) understood that the consent included an authorisation to access to medical files.

The interviews pointed out that while contribution to biobanks is considered an act of solidarity and reciprocity, persons were more reluctant to consider financial issues. Most of the patients (82.4%) would accept that a biological sample could be given to another public laboratory. This rate was higher than considering a transfer to a private laboratory (62.9%; $p <0.001$). Acceptance that tumour samples could be sold were very low for both types of laboratories even more for a public laboratory (10.6% vs 13.2%; $p=0.017$).

Conclusion: Quality of biobank informed consent should be improved. Specific information should be provided on the potential transfer of samples and associated clinical data for large-scale multicentric research. Education should also stress financial issues in science, for the purposes of transparency and efficacy.

C11.6 Biobanking cancer tissue. Patients consider excised (tumour) tissue to be 'connective tissue'.

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Background Excised tissues are routinely stored in hospitals for future diagnostic use. These tissues are also important for scientific research. In the Netherlands 'residual tissues' are registered in a central pathological registry (PALGA), which makes this collection, consisting of millions of samples, a very large biobank.

Aim The presentation elucidates and analyses the underlying attitude of patients towards medical research with excised tissues and focuses on the use of the tissue in genetic medical research. Concepts of ownership in the context of increasing (commercial) value of tissues are discussed.

Methods The study is based on mixed methods design combining quantitative data (questionnaires) with qualitative data (transcripts from interviews) and observations during an intervention study at the Netherlands Cancer Institute. In total 260 patients were interviewed, 61% of 426 patients who completed a written questionnaire.

Results Many (38%) patients considered themselves to be owner of stored tissue and 43% considered themselves owner of DNA in stored tissue. Most, 98%, endorse medical research with this tissue. For patients the stored tissue is 'a hypercollective good' that should remain in the public sphere in order to facilitate research. Patients consider the tissue to be connective because it connects them to others through research. Patients expect to be reciprocated by the tissue holder and be informed about findings of the research.

Conclusion Information about medical research with residual tissue should be improved. A more participatory and reciprocal model of biobanking is required.

C12.1 Homozygosity Mapping of Primary Microcephaly in 112 Iranian Families: Novel Mutations and Phenotypes

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Primary microcephaly (MCPH) is a genetically heterogeneous disorder showing an autosomal recessive mode of inheritance. Affected individuals present with head circumferences more than three standard deviations below the age- and sex-matched population mean, accompanied by mental retardation without further associated malformations. Five genes (*mcph1*, *cdk5rap2*, *aspm*, *cenpj*, *STIL*) and two genomic

loci, MCPH2, and 4 have been identified so far.

In this study, we investigated all seven loci in patients with primary microcephaly from 112 Iranian families. In addition to a thorough clinical characterization, karyotype analyses were performed for all patients. For linkage analyses, several microsatellite markers were selected for each locus and used for genotyping.

Our investigation enabled us to detect linkage to the MCPH5 (*ASPM*) region in thirteen families. Three families showed linkage to MCPH2, eight to MCPH1 (*Microcephalin*), five to MCPH6 (*CENPJ*) and two families were linked to MCPH7 (*STIL*). The remaining 81 families were not linked to any of the seven known loci. Subsequent sequencing revealed 10 novel mutations and one previously reported mutation in *ASPM*, 8 novel mutations in *MCPH1* as well as a novel mutation in *CENPJ*.

In some families, additional features such as short stature, seizures or congenital hearing loss were observed in the microcephalic patients, which widens the spectrum of clinical manifestations of mutations in known microcephaly genes.

Our results show that the molecular basis of microcephaly is more heterogeneous in Iran than elsewhere, thus the Iranian population provides a unique opportunity for finding additional genes underlying this disorder.

C12.2* Combination of linkage mapping and microarray-expression analysis identifies NF- κ B signalling defect as a novel cause for autosomal recessive mental retardation

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Autosomal recessive inheritance accounts for nearly 25% of non-syndromic mental retardation (MR) but the extreme heterogeneity of these conditions markedly hampers gene identification. Combining autozygosity mapping and RNA expression profiling in a consanguineous Tunisian family of three MR children with mild microcephaly and white matter abnormalities identified the NIBP/TRAPPC9 gene, which encodes a NF- κ B inducing kinase (NIK) and I κ B kinase complex β (IKK β) binding protein, as a likely candidate. Sequencing analysis revealed a nonsense variant (c.1708C>T, p.R570X) within the exon 9 of this gene responsible for an undetectable level of NIBP protein in patient skin fibroblasts. Moreover, TNF- α stimulation assays showed a defect in I κ B α degradation, suggesting an impaired NF- κ B signalling in patient cells. This study provides the first evidence of a NF- κ B signalling defect in isolated MR.

C12.3* Targeted next generation sequencing identifies a mutation associated with cerebellar hypoplasia and mental retardation with quadrupedal locomotion

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We describe the utilization of next generation sequencing in the identification of a mutation associated with a rare and heterogenous Mendelian disorder characterized by cerebellar hypoplasia, mental retardation and quadrupedal locomotion (Uner Tan syndrome). Homozygosity mapping, sequence capture (Nimblegen) of the disease locus on chromosome 17p13 and massively parallel DNA sequencing (454 GS FLX) of two affected and two carrier individuals yielded 362 to 403 million base pairs of sequence information from each sample. Between 96.6%-96.7% of all reads fell within the minimal critical region. Variants common to all samples were selected and binned into four groups (G): variants in coding regions, consensus splices-sites and RNA genes are G-1 (n=26), in 5'UTRs & 3'UTR s are G-2 (24), in introns are G-3 (929). All remaining variants were classified as G-4 (577) (Table-1). A total of 17 novel exonic variants (four non-synonymous, two synonymous, one 5'UTR and ten 3'UTR) compatible with the expected autosomal recessive inheritance model of the disease allele was observed (Mendelian compatibility). An exclusion based population screening approach was adopted to distinguish the disease causing variant from

novel polymorphisms by genotyping all non-synonymous variants in 300 healthy controls. All but one variant was observed in the controls with allele frequencies of 0.003, 0.005, 0.016. These results indicate that next generation sequencing is a powerful tool to identify mutations associated with rare diseases.

Table 1. Direct identification of a disease-associated mutation by next generation sequencing.

| | Carrier mother | Carrier father | Affected child 1 | Affected child 2 |
|--|----------------|----------------|------------------|------------------|
| Number of mapped bases | 403,546,905 | 362,750,092 | 410,247,834 | 399,184,755 |
| Mean coverage | 48.4x | 40.5x | 47.4x | 48.9x |
| Coverage of targeted regions (≥5) | 99.3% | 99.2% | 99.3% | 99.2% |
| All variants | 26,751 | 27,471 | 24,115 | 24,690 |
| dbSNP129 | 10,564 | 10,694 | 8,200 | 8,289 |
| Novel variants | 16,157 | 16,777 | 15,915 | 16,401 |
| High confidence variants | | | | |
| Heterozygous SNPs | 4,874 | 5,076 | 408 | 596 |
| Homozygous SNPs | 4,647 | 4,523 | 7,095 | 7,563 |
| Novel heterozygous variants | 1,331 | 1,600 | 513 | 458 |
| Novel homozygous variants | 1,090 | 1,602 | 1,789 | 2,029 |
| Common variants to all samples | | | | |
| G-1 (coding, splice site, RNA) | | | 26 | |
| G-2 (5' and 3'UTR) | | | 24 | |
| G-3 (Intronic) | | | 929 | |
| G-4 (Intergenic) | | | 577 | |
| G-1 Mendelian compatibility | | | 4 | |
| After population screening | | | 1 | |

C12.4* The Arg164X mutation in *SAMHD1* leads to a novel variant of Aicardi-Goutières syndrome by modulating cytokine expression

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Aicardi-Goutières syndrome (AGS) is a rare inborn multisystemic disease, resembling intrauterine viral infection resulting in psychomotor retardation, spasticity and chilblain-like skin lesions. Diagnostic criteria include intracerebral calcifications and elevated interferon-alpha and pterins in cerebrospinal fluid. Patients present early in infancy and death usually occurs during childhood in a state of decerebration. We report on four adult siblings of Turkish origin with unknown neuro-degenerative disease presenting with stenoses of intracranial vessels, stroke and glaucoma in childhood, two of whom died at the age of 40 and 29 years. Genome wide homozygosity mapping identified 170 candidate genes embedded in a common haplotype of 8Mb on chromosome 20q11-13. Next generation sequencing of the entire region identified the c.490C>T (p.Arg164X) mutation in *SAMHD1*, a gene most recently described in AGS, on both alleles in all affected siblings. Clinical diagnosis of AGS was then confirmed by demonstrating intracerebral calcifications on cranial computed tomography and elevated pterin levels in cerebrospinal fluid in the two surviving siblings. In patient fibroblasts *SAMHD1* protein was undetectable, while basal expression of interleukin-8 was increased and stimulated expression of interferon- β was reduced. We conclude that the Arg164X mutation in *SAMHD1* by modulating intravascular cytokine expression leads to a novel phenotypic variant of AGS.

C12.5 Systematic mutation search in families with X-linked mental retardation by next-generation sequencing

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X-linked mental retardation (XLMR) affects 1-2/1,000 males and accounts for 10% of all forms of mental retardation. Recent screening of X-linked genes has revealed truncating mutations in 25% of the families studied (Tarpey et al, 2009). Previous studies had indicated that mutations in known XLMR genes account for at least 42% of XLMR families (de Brouwer et al, 2007). To resolve this discrepancy and to shed more light on the molecular causes of XLMR, we have combined genome partitioning techniques and Next Generation Sequencing (NGS) to find the causative gene defect in another 200 families from the European MRX Consortium.

Here we report the results on the first 100 families. About 25% of the families carried potentially pathogenic mutations in known XLMR genes. Many other families carried non-synonymous, possibly pathogenic changes in novel genes, but only 3 pathogenic truncating mutations were observed. There are several plausible explanations for this: prior to these investigations, many of these large families had already been screened for mutations in known XLMR genes and positional candidates. Secondly, the EuroMRX cohort is enriched for families with non-syndromic XLMR, and it is conceivable that complete loss of function will often result in severe syndromic forms or even lethality. Thus, the majority of genes whose loss gives rise to non-syndromic XLMR may already be known. Finally, the fundamental defect may not reside in coding regions of the X-chromosome. Sequencing of entire sorted X-chromosomes is being performed to shed more light on these issues.

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C12.6 Deep sequencing leads to the identification of 3 independent mutations in the ST3GAL3 gene in patients with autosomal recessive intellectual disability from 3 consanguineous Iranian families

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Most mutations causing intellectual disability (ID) are thought to affect autosomal genes, yet to date only six are known to play a role in the etiology of autosomal recessive ID (ARID). By identifying 12 additional loci in 78 families with non-syndromic ARID (NS-ARID) we have previously shown, that the molecular basis of NS-ARID is extremely heterogeneous (Najmabadi et al., 2007, Hum.Genet. 121(1):43-8). Indeed, only twice have more than one independent NS-ARID causing mutation so far been found to affect the same gene (TUSC3, TRAPPC9). We have now identified three consanguineous families where linkage analysis yielded overlapping homozygous intervals on chromosome 1p34. Using Chromosome sorting to enrich Chr1 from lymphoblastoid cell lines of patients from two of these families followed by deep sequencing, we found two different missense mutations affecting the ST3GAL3 gene. Subsequent screening of ST3GAL3 in the remaining family revealed an additional mutation with a putatively damaging splicing effect. All three changes co-segregate with the disease and were absent in more than 150 controls. This supports the assumption that ST3GAL3 might have a considerably increased mutation frequency in ARID, at least in Iran. The ST3GAL3 gene product is a type II membrane protein, which catalyzes sialic acid transfer from CMP-sialic acid to galactose-containing substrates. Together with colleagues from the Hannover Medical School (M. Muehlenhoff et al.), we are presently investigating the impact of the mutations on ST3GAL3 protein function to understand the pathology of MR in these patients and thus also gain new insights into human brain function.

C13.1 A recurrent 14q32.2 microdeletion mediated by expanded TGG repeats

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Nearly all recurrent microdeletion/duplication syndromes described to date are characterized by the presence of flanking low copy repeats that act as substrates for non-allelic homologous recombination (NAHR) leading to the loss, gain or disruption of dosage sensitive genes. We describe an identical 1.11 Mb heterozygous deletion of 14q32.2 including the *DLK1/GTL2* imprinted gene cluster in two unrelated patients. In both patients the deleted chromosome 14 was of paternal origin, and consistent with this both exhibit clinical features compatible with UPD(14)mat. Using a high-resolution oligonucleotide array we mapped the breakpoints of this recurrent deletion to large flanking (TGG)_n tandem repeats, each approximately 500bp in size and sharing $\geq 88\%$ homology. These expanded (TGG)_n motifs share features with known fragile sites and are predicted to form strong guanine quadruplex secondary structures. We suggest that this recurrent deletion is mediated either by NAHR between the TGG repeats, or alternatively results from their inherent instability and/or strong secondary structure. Our results define a recurrent microdeletion of the 14q32.2 imprinted gene cluster mediated by flanking (TGG)_n repeats, identifying a novel mechanism of recurrent genomic rearrangement. Our observation that expanded repeats can act as catalysts for genomic rearrangement extends the role of triplet repeats in human disease, raising the possibility that similar repeat structures may act as substrates for pathogenic rearrangements genome-wide.

C13.2 Recurrent Chromosomal Translocations Mediated by Genomic Interchromosomal NAHR

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Sequence analysis of the breakpoints were performed in 4 unrelated families with the same unbalanced translocation der(4)(t(4;11)(p16.2;p15.4) and revealed the presence of large 200-300 kb low copy repeats (LCRs) on 4p16.2 and 11p15.4 of 94% interchromosomal sequence identity. The breakpoints for both the short arms of chromosomes 4 and 11 were mapped within the homologous subunits; consistent with nonallelic homologous recombination (NAHR) as the mechanism for translocation. To investigate the potential involvement of interchromosomal LCRs in recurrent chromosomal translocation formation, we performed computational genome-wide analysis and identified 470 interchromosomal LCRs substrate pairs, greater than 30 kb in size and sharing $>94\%$ sequence identity, that may mediate chromosomal translocations. Several predicted recurrent translocations were identified, initially at the G-band level of resolution from clinical cytogenetic databases; in two translocations, t(4;8)(p16.1;p23.1) and t(8;12)(p23.1;p13.31), the breakpoints of seven cases and two cases, respectively, were mapped molecularly to the predicted LCRs. We show that interchromosomal LCRs in 11p15.4 mediate the recurrent constitutional translocation t(4;11)(p16.2;p15.4) via NAHR, provide a computationally determined "recurrent translocation map" and demonstrate its utility, and further suggest that NAHR may mediate recurrent translocations throughout the human genome.

C13.3 The interpretation of copy number gains detected by high resolution array CGH in routine diagnostics; a practical guideline

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The introduction of high resolution array Comparative Genomic Hybridisation (CGH) has led to the detection of large numbers of copy number variations (CNVs) in patients with developmental delay and/or multiple congenital anomalies (DD/MCA) as well as in healthy individuals. The detection of a CNV in DD/MCA does not automatically imply a phenotypic effect. The interpretation of copy number gains is even more complicated than of copy number losses, because of the milder and more variable phenotype associated with microduplications. The aim of the present study was to develop a guideline for the interpretation of gains detected by array-CGH.

All copy number gains of at least 4 adjacent oligonucleotides but less than 10 Mb in size, detected in 300 consecutive patients analysed by custom made 105K Agilent oligo array were collected and the clinical relevance was evaluated using an interpretation scheme.

Of a total of 797 gains, 45% were *de novo* and 55% were familial. 95% of all *de novo* and 87% of all familial gains were concluded to be benign CNVs. Fifteen pathogenic gains, sized 288-7,912 kb, were detected. These gains were significantly larger than benign gains and gains of unknown clinical relevance ($p < 0.001$). Surprisingly, they were more often familial (10/14) than *de novo* (4/14) (not statistically significant).

A threshold of 200 kb for the detection of gains in routine diagnostics was concluded to be satisfactory at the moment. A practical guideline to interpret copy number gains was formulated and validated using an independent patient cohort. This guideline will be discussed.

C13.4 Paternal origin of *de novo* constitutional t(11;22)(q23;q11)

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The constitutional t(11;22)(q23;q11) is a well-known recurrent non-Robertsonian translocation in humans. While translocations generally occur in a random fashion, the breakpoints of t(11;22)s are concentrated within several hundred base pairs on 11q23 and 22q11. These regions are characterized by palindromic AT-rich repeats (PATRRs), which could cause the genomic instability. *De novo* t(11;22)s are detected in sperm from healthy males at a frequency of $1/10^4$ - 10^5 , but never in lymphoblasts, fibroblasts or other human somatic cell lines, suggesting that the generation of a t(11;22) is linked to gametogenesis. However, female germ cells have not been tested since the number of human oocytes that can be examined is limited. To investigate whether the translocation is gametogenesis-specific or male germ cell specific, we attempted to determine parental origin of *de novo* t(11;22) cases. We studied 8 carriers of *de novo* t(11;22)s and their parents. The PATRRs and flanking regions on chromosome 11, 22, der(11) and der(22) were amplified by PCR and the nucleotide sequences were determined. The highly polymorphic nature of the PATRRs allowed us to determine the parental origin of the *de novo* t(11;22)s. All of the eight translocations were found to be of paternal origin. This result implicates a possible novel mechanism of sperm-specific generation of t(11;22)s. It is proposed that replication errors during the numerous cell divisions in pre-meiotic spermatogenic cells or conformational changes of the DNA during chromatin remodeling in the post-meiotic stages of spermatogenesis might contribute to male-specific formation of *de novo* t(11;22)s.

C13.5 ALT-immortalized human cells are critically dependent on the Fanconi anemia protein FANCD2 to limit BLM-dependent recombination and amplification of telomeric repeat DNA

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Fanconi anemia (FA) is an inherited disorder characterized by bone marrow failure, cancer predisposition and congenital malformations. FA proteins are implicated in homologous recombination (HR), a process involved in Alternative Lengthening of Telomeres (ALT) pathways. The FA protein FANCD2 interacts with other HR proteins, however the precise role of FANCD2 in HR is unclear. We now find FANCD2's ability to limit BLM-dependent telomeric recombination and amplification events is critical for human ALT cell telomere maintenance and survival.

FANCD2 visibly localizes to telomeric foci and PML bodies in ALT, but not in telomerase-positive cells. FANCD2 almost always localizes to telomeric foci containing BLM, and telomeric localization of FANCD2 is BLM-dependent. FANCD2 depletion results in ALT-specific telomere dysfunction characterized by increases in telomeric DNA synthesis, entanglements, recombination events, and association with DNA damage response proteins. Amplified telomeric DNA in FANCD2-depleted cells is primarily extrachromosomal, accumulating both outside of and within PML bodies. We previously reported that BLM overexpression causes a similar phenotype of rapid, large scale ALT-specific amplification of telomeric DNA. Co-depletion of BLM with FANCD2 completely suppresses the telomere phenotypes caused by FANCD2 knockdown, suggesting that the FANCD2-depletion phenotype requires functional BLM. In contrast, co-depletion of RAD51 with FANCD2 does not suppress the FANCD2-depletion phenotype, suggesting that human ALT is RAD51 independent.

We propose that FANCD2 restrains BLM-dependent, RAD51-independent recombination and amplification of telomeric DNA in ALT cells by limiting production of ssDNA gapped regions in telomeric DNA, and/or by affecting stability of recombination/replication intermediates.

C13.6 Delineating complex genomic architecture involving chromosome segmental duplications

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Complex genomic architectures predispose to chromosomal rearrangements. The rearrangements can cause a diverse range of phenotypes due to haploinsufficiency or positional effects from genes in *cis* with families of segmental duplications (SDs). Genomic regions flanked by SDs in Angelman and Prader-Willi syndromes (AS/PWS) show a great proclivity for such chromosomal structural changes. The most common rearrangements in AS/PWS involve large (Class I) and small (Class II) deletions sharing a common telomeric region of breakage. FISH can detect juxtaposed DNA sequences in normal and abnormal chromosomes which can delineate the locations of these sequences within individual SD elements based on differences in their respective genomic contexts. In this study, we computationally designed and developed 47 single copy (sc) and low copy (lc) sequence-defined FISH probes to delineate proximal and distal ends of the chromosome 15q11-q13 deletion in Class I and Class II deletions. Departure from the expected fluorescent signals using a two-step hybridization algorithm directed which sc and lc FISH probe combinations were used in subsequent hybridizations. *In situ* analysis demonstrated variations in deletion extent at both the proximal and distal ends within each patient group. Delineated genomic intervals suggest that SDs of both high and low sequence homologies as well as regions adjacent to SD blocks are giving genesis to the dynamic breakage activity in this highly unstable region. These strategies should be useful for delineation of rearrangement boundaries for other disorders associated with SDs and can provide unifying mechanisms by which complex genomic architectures cause instability in human chromosomes.

C14.1 Dysostin, a new gene involved in CDG and affecting pH homeostasis

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The correct structure of the oligosaccharides on glycoproteins depends on the enzymatic activities of numerous glycosyltransferases and remodeling glycosidases, but also on a precise Golgi localization of these enzymes and an adequate intracellular environment. Genetic defects affecting this glycosylation pathway cause a range of diseases known as Congenital Disorders of Glycosylation (CDG).

Through a combination of homozygosity mapping and expression analysis in fibroblasts, we have been able to identify a novel gene involved in CDG in a single, nuclear family, and pinpoint the defect to a deep intronic mutation. We subsequently identified missense mutations in 2 other, unrelated cases. Because of the patients' phenotype, we named it 'dysostin'.

In the patients' fibroblasts, a dilated Golgi morphology associated with

a fragmentation of the trans Golgi network was observed. A slight delay in the retrograde translocation of Golgi membranes to the endoplasmic reticulum was detected after treatment with brefeldin A, suggesting that intracellular trafficking is compromised. Co-localization of the fluorescently-tagged protein with relevant markers revealed a late endosomal/lysosomal localization, which is peculiar in view of the glycosylation defect. pH measurements in late endocytic structures using the Lysosensor dye revealed a higher staining - and hence a more pronounced acidity - in those compartments in the patients. This result was reproducible in HeLa cells, using a siRNA strategy to knockdown dysostin. In summary, the powerful combination of genetic and expression studies allowed us to discover a novel CDG gene; the cellular assays revealed completely novel connections between lysosomal pH, glycosylation and intracellular trafficking.

C14.2 A new inborn error of glycosylation due to DPM2 deficiency

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Congenital Disorders of glycosylation (CDG) are a group of complex metabolic diseases. About 40 types have been identified. Most CDG with a type I pattern on transferrin isoelectric focusing (CDG-I) result from defects in the glycosyltransferases and sugar transporters, needed for glycan assembly in the endoplasmic reticulum.

We report on a CDG-I patient with severe developmental delay, epilepsy and dysmorphic features. Analysis of the lipid-linked oligosaccharides (LLO) showed an accumulation of dol-PP-GlcNAc2-Man5. This pattern is compatible with a defect in DPM1, ALG3 or MPDU1, in which mutations have been described; however, no mutations were detected. We decided to further analyze the DPM2 and SAC1 genes. The patient was found to be compound heterozygote for 2 mutations in DPM2: a splice mutation (c.4-1G>C) and a missense mutation (c.68A>G, p.Y23C).

Hence, this patient presents with a novel type of CDG. The human dolichol-phosphate-mannose (DPM) synthase is a heterotrimeric complex composed of DPM1, DPM2 and DPM3. Until now, only mutations in DPM1 (the catalytic subunit) and more recently in DPM3 have been described. DPM2 is a hydrophobic protein of 84 amino acids, whose function is still not clear. It is reported in the literature that DPM2 could be involved in the regulation of the DPM synthase complex but also in the regulation of the glycosylphosphatidylinositol-N-acetylglucosaminyltransferase, and a defect in DPM2 may also affect GPI-anchored proteins - a feature which has not been studied so far in CDG patients. Further investigation of the DPM2 mutations by complementation in Lec15 hamster DPM2-deficient cells is ongoing.

C14.3* A single-nucleotide deletion in the POMP 5' UTR causes a transcriptional switch and an altered epidermal proteasome distribution in KLICK genodermatoses

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KLICK syndrome is a rare autosomal recessive skin disorder characterized by palmoplantar keratoderma, linear hyperkeratotic papules over joints, sclerotic constrictions around fingers and ichthyosiform scaling. We identified twelve cases, from five European countries, that share the specific KLICK manifestations. Using genome-wide SNP-based homozygosity mapping we identified a 1.5-Mb homozygous candidate region on chromosome 13q. Sequence analysis of

the ten annotated genes in the candidate region revealed homozygosity for a single-nucleotide deletion at position c.-95 in the proteasome maturation protein (*POMP*) gene, in all patients. The deletion was associated with a shift in expression of *POMP* transcript variants with a marked increase of transcripts with elongated 5' untranslated regions (UTRs) in keratinocytes. *POMP* functions as a chaperone for proteasome maturation and immunohistochemical analysis of skin biopsies from KLICK patients revealed an altered distribution of *POMP*, the proteasome subunit proteins α 7 and β 5 and the ER stress marker CHOP in the most differentiated skin layers. Furthermore, the KLICK patients showed a deviant expression of the skin differentiation marker filaggrin. Our results suggest that KLICK syndrome is caused by a single-nucleotide deletion in the 5' UTR of *POMP*, resulting in altered distribution of *POMP* and proteasomes in epidermis and a perturbed formation of the outermost layers of the skin. These findings imply that the proteasome has a prominent role in the terminal differentiation of human epidermis and that elevated ER stress is a disease mechanism in genodermatoses.

C14.4* Mutation in SHOC2 promotes aberrant protein N-myristoylation and underlies Noonan-like syndrome with loose anagen hair

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N-myristoylation is a common form of co-translational protein fatty acylation resulting from the attachment of myristate to a required N-terminal glycine residue. We show that aberrantly acquired N-myristoylation of SHOC2, a leucine-rich repeat-containing protein that positively modulates RAS-MAPK signal flow, underlies a clinically distinctive condition of the neuro-cardio-facial-cutaneous disorders family. Twenty-five subjects with a relatively consistent phenotype previously termed Noonan-like syndrome with loose anagen hair [OMIM 607721] shared the 4A>G missense change (Ser2Gly) in SHOC2 that introduces an N-myristoylation site, resulting in aberrant targeting of SHOC2 to the plasma membrane and impaired translocation to the nucleus upon growth factor stimulation. Expression of SHOC2^{S2G} in vitro enhanced MAPK activation in a cell type-specific fashion. Induction of SHOC2^{S2G} in *Caenorhabditis elegans* engendered protruding vulva, a neomorphic phenotype previously associated with aberrant signaling. These results document the first example of an acquired N-terminal lipid modification of a protein causing human disease.

C14.5* Another gene for autosomal recessive ALX-related frontonasal dysplasias: Disruption in ALX1 (CART1) causes anophthalmia and severe facial clefting

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Molecular genetics of recessively inherited frontonasal dysplasias (FNDs) are largely unknown. In 2009, two studies have drawn attention to the critical role of aristotels-related homeobox transcription factors, *ALX3* and *ALX4*, in the molecular pathogenesis of autosomal recessive-FND in humans (Am. J. Hum. Genet. 2009; 84: 698 and Hum Mol Genet. 2009; 18: 4357, respectively). We present a new autosomal recessive frontonasal dysplasia in two families characterized by bilateral anophthalmia/microphthalmia, bilateral oblique facial cleft, complete cleft palate, hypertelorism, wide nasal bridge with hypoplasia of ala nasi, and low set and posteriorly rotated ears. Using Affymetrix 250K SNP Array genotyping and homozygosity mapping, we mapped this clinical entity to chromosome 12q21. In one of the families with three affected sibs, CNV analysis of the critical region detected a homozygous 3.7 Mb deletion containing the *ALX1* (*CART1*) gene. In the second family, we identified a homozygous donor splice site mutation (c.537+1 G>A), which is predicted to disrupt the functionally essential homeodomain structure of the *ALX1* protein. These results provide evidence that loss of *ALX1* function causes severe impairment of early craniofacial development. Unlike its murine ortholog, complete loss of human *ALX1* does not result in neural tube defects, however, severely affects the orchestrated fusions between frontonasal, nasomedial, nasolateral, and maxillary processes in early embryogenesis. This study further expands the spectrum of the recently recognized autosomal recessive "ALX-related FND" phenotypes in humans. The study was done within the CRANIRARE consortium supported by the European Research Area Network, "E-RARE" through Turkish Scientific Council.

C14.6* High-throughput mutation screening in combination with cellular complementation of rare variants aid gene identification in mitochondrial disorders

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We performed a multi-centre large-scale mutation screen of 70 candidate genes in 150 patients with isolated complex I deficiency by high resolution melting curve analysis and Sanger sequencing.

For the first time we identified pathogenic mutations in the mitochondrial respiratory chain complex I subunit NDUFB9. Additionally, we identified causative mutations in another 16 genes, which have previously been associated with complex I deficiency: 5 mtDNA encoded subunits, 3 tRNA genes, 6 nuclear encoded subunits and 3 assembly factors. The causality of newly identified missense mutations was established by complementation of complex I activity in patient-derived fibroblast cell lines by lentiviral expression of wildtype cDNA.

We have initiated an exome sequencing approach starting with familial cases to identify mutations in the 70% of patients with no mutations found yet. Taking advantage of the complementation approach, we will be able to discriminate pathogenic from non-pathogenic variants.

C15.1* Genome-wide association study of regional brain volume suggests involvement of known psychiatry candidate genes, identifies new candidates for psychiatric disorders and points to potential modes of their action

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Psychiatric diseases are highly heritable disorders with complex etiology partially explained by combinations of genes with small effect size interacting with each other and with environmental factors. The small effect sizes of genetic risk factors have hampered the identification of genes predisposing to these diseases. A possible way to aid identification of risk genes is the use of intermediate phenotypes. These can be defined e.g. at the level of brain function and of regional brain structure. Both are highly heritable, and regional brain structure is linked to brain function.

Within the Brain Imaging Genetics (BIG) study at the Radboud University Nijmegen (Medical-Centre) we performed a genome-wide association study (GWAS) in 600 of the currently 1400 healthy study participants. For all BIG participants, structural MRI brain images were available. Gray and white matter volumes were determined by brain segmentation using SPM software. FSL-FIRST was used to assess volumes of specific brain structures. Genotyping was performed on Affymetrix 6.0 arrays.

Known candidates from studies on psychiatric genetics and mental disorders are implicated in the regulation of regional brain structure. E.g., *CDH13*, associated with ADHD, schizophrenia and substance abuse, was associated with amygdala, hippocampus and white matter volumes ($P<10E-05$); *CANA1C*, associated with bipolar disorder, was found associated with brainstem volume ($p<10E-05$) and three SNPs on the *PLXNA2* gene showed p -values $<10E-05$ with caudate nucleus volume.

Our data suggests that genes involved in psychiatric and mental disorders are also associated with the variance of intermediate phenotypes based on brain volumes from healthy individuals.

C15.2 NXF/ARNT2/SIM2, RET Expression regulation and specific HSCR Associated DNA Variants

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Two non-coding *RET* variations, the T allele of SNP rs2435357 (Enh1-T) and the A allele of SNP rs2506004 (Enh2-A) proved strongly associated with Hirschsprung (HSCR) susceptibility. Furthermore, in two SNPs are in strong linkage disequilibrium and both located in an enhancer element in intron 1 of the *RET* gene. For Enh1 it has been shown that the disease associated T allele results in reduced expression in Luciferase experiments, via reduced SOX10 binding, when compared to non-disease associated C allele. The goals of this study were to determine whether Enh2-A also is a functional variant, i.e. affect *RET* expression. We generated reporter constructs containing both alleles of the two SNPs, separately or in combinations, coupled to the Luciferase gene. Luciferase assays showed that not only the Enh1-T allele but also the Enh2-A allele decreased reporter gene, showing that both SNPs do contribute independently. *MatsInspector* software identified the sequences of Enh2-C (non-disease associated variant) and its surroundings sequences (-ACGTG-) as a potential binding site for the (heterodimer) transcription activator NXF/ARNT2, and the (heterodimer) transcription repressors SIM2/ARNT2. Binding affinity of NXF/ARNT2 to Enh2-C was confirmed by Electrophoresis Mobility Shift Assays and Supershift. Transfections of NXF/ARNT2 or SIM2/ARNT2 into Neuroblastoma cell lines increase and decrease *RET* expression respectively as expected. Interestingly SIM2 is located on chromosome 21 and trisomy 21 strongly increases the risk on HSCR. Most importantly, our data shows that more than one SNP on an associated haplotype might influence disease development making polygenic diseases even more complex than originally thought

C15.3 Genomewide association of single nucleotide polymorphisms with subcutaneous adipose tissue gene expression.

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Exploration of the genetic control of gene expression in adipose tissue could provide valuable insights into the genetic architecture of common human obesity. Using whole genome microarrays, a genome wide association analysis was carried out using gene expression levels measured in subcutaneous adipose tissue samples from obesity discordant sibpairs as quantitative traits.

The SOS SibPair cohort was utilised, which consists of 154 nuclear families (n=732), each containing an obesity-discordant sib pair (Body Mass Index difference ≥ 10 kg/m²). RNA was extracted from subcutaneous adipose tissue and DNA was extracted from blood. Gene expression and genotyping data was generated from all 349 siblings using the Affymetrix Human U133 Plus 2.0 and Illumina Human 610K arrays, respectively. Analysis of the genomewide gene expression and genotype data was carried out using Merlin.

Approximately 10% of all transcripts (~5500) were associated with SNP markers in the genome, either in *cis* or in *trans*, using the very stringent threshold of a Bonferroni correction of $p=3.6 \times 10^{-12}$. For a False Discovery Rate of 10%, 4400 transcripts differentially expressed between lean and obese siblings were detected. Using a Bonferroni correction of $p=1 \times 10^{-7}$, approximately 350 of the differentially expressed transcripts were also *cis*-eQTLs and approximately 150 were also *trans*-eQTLs. In the next stage, differential expression analysis and incorporation of the obesity status in the association framework will allow the identification of SNP markers and pathways involved in obesity susceptibility.

C15.4 Mutations of VANGL1 in patients with neural tube defects

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Neural tube defects (NTD) are congenital malformations resulting from failure of neurulation. Recent years witnessed a breakthrough in elucidating the role of planar cell polarity (PCP) pathway in neurulation and how molecular lesions in this pathway lead to NTD in animal models and humans. PCP is controlled by the non canonical *Fz/Dvl* signalling pathway that involves a number of additional core gene, including *Stbm/Vang*, *Fmi*, *Pk*, and *Dgo*. The *Loop-tail* (*Lp*) mouse that develops craniorachischisis carry missense mutations in the *Vangl2* gene, that is the mammalian homolog of the *Drosophila Stbm/Vang* gene required for establishing planar cell polarity in many tissues. This mouse provided the first line of evidence for involvement of PCP pathway in NTD in mammals. *Vangl1*, a vertebrate homolog of *Vangl2*, encodes for a transmembrane protein containing a PDZ-domain binding motif involved in protein-protein interactions. We sequenced human *VANGL1* gene in a cohort of 810 NTD patients and we identified 8 missense mutations both in familial (V239I, R274Q, S83L, and R181E) and sporadic (M238T, F153S, L202F, and A404S) cases. These mutations affect highly conserved residues and were not found in 1200 controls. We demonstrated that V239I mutation abrogates *in vitro* the interaction between VANGL protein and DVL, strongly suggesting a pathogenic effect on the protein function. Finally, we demonstrated that two human mutations, V239I and M238T, affect convergent extension in zebrafish and we hypothesize that they most likely affect a similar process in humans. These results support a role for *VANGL1* as genetic risk factor for NTD.

C15.5 Functional analysis of fatty acid desaturase (FADS) gene cluster polymorphisms

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Fatty acid desaturases play a pivotal role in the endogenous formation of n-6 and n-3 long-chain polyunsaturated fatty acids (LC-PUFAs). Several studies reported associations of single nucleotide polymorphisms (SNPs) in the human desaturase encoding genes (FADS1, FADS2) with LC-PUFA levels, with an amazingly high genetically explained variance (28.5%) for arachidonic acid. Moreover, SNPs in this cluster were associated with more complex phenotypes such as cholesterol and triglyceride levels as well as glucose levels. The functional relevant SNP(s) could not be identified by these association studies, because all associated SNPs are in high linkage disequilibrium. The aim of our study is therefore to identify the causative variant(s) to learn more about the regulatory pathways involved in LC-PUFA homeostasis.

We identified two interesting polymorphisms (rs3834458 and rs968567) in the FADS2 gene promoter by bioinformatics analysis. Luciferase reporter gene assays revealed that the minor T allele of SNP rs968567 leads to a significant increase in promoter activity in cell lines, whereas rs3834458 showed no significant effect. Competitive electrophoretic mobility shift assays showed allele-specific binding of nuclear proteins for the rs968567 surrounding region, but no differential results were obtained for rs3834458. One of the proteins binding to the rs968567 surrounding region in an allele-specific manner was identified as transcription factor ELK1. Altogether, our results indicate that rs968567 influences FADS2 transcription, probably by genotype dependent ELK1 binding, and offer first insights into modulation of FADS2 gene transcription by SNPs.

C15.6 Mutation and functional analysis of the IRAK-M gene in asthmatic patients.

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Asthma is a complex disease caused by the interaction between genes and environment. Since its prevalence and associated mortality is largely increasing in the latest years, there is a great interest in understanding the molecular basis of this pathology. In 2007 we identified IRAK-M as an asthma susceptibility gene in the Sardinian population and replicated the study in two genetically distant populations. To better understand the role of IRAK-M in the pathogenesis of asthma, we first conducted a mutation screen in affected individuals and in controls to identify the functional variations responsible of the association. Our analysis revealed a clustering of rare coding mutations in patients and functional studies in different cell lines showed that some affect downstream activation of NF- κ B, a transcription factors involved in innate immunity and inflammation negatively regulated by IRAK-M. Combining bioinformatics analysis and deletion studies in cell lines, we identified a region upstream of IRAK-M with promoter activity, containing several binding sites for transcription factors known to modulate inflammatory responses (c-REL, AP1, NFAT). EMSA experiments for the predicted binding sites demonstrated specific binding in monocyte cell lines stimulated with LPS, whereas site directed mutagenesis showed that destruction of the binding sites greatly reduced activity of the IRAK-M promoter region. These data further support a potential role of IRAK-M in the pathogenesis of chronic inflammation and asthma.

C16.1 The revised Ghent nosology for the Marfan syndrome (MFS)

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The diagnosis of MFS relies on a set of international criteria, outlined by expert opinion. In 1996, the initial Berlin nosology was redefined because of the risk of overdiagnosis into the Ghent nosology, a more stringent set of major and minor criteria.

These Ghent criteria have proven to work well since with improving molecular techniques, confirmation of the diagnosis is possible in over 95% of patients. However, concerns with the Ghent criteria are that some of the diagnostic manifestations have not been validated as thresholds and others necessitate cumbersome imaging studies. Moreover, in the absence of aortic dilation, the diagnosis can be stigmatizing, hamper career aspirations and restrict life-insurances opportunities. The label "MFS" may cause psychosocial burden by "restricted exercise permission".

Following an international expert meeting, we propose a revised Ghent nosology in which aortic root aneurysm and ectopia lentis are cardinal features. In absence of family history, the presence of these two manifestations is sufficient for the unequivocal diagnosis of MFS. In absence of any of these two, the presence of bona fide FBN1 mutation or a combination of systemic features is required. For the latter a new scoring system has been designed and validated. In this way FBN1 testing is not mandatory but useful when available.

The proposed new nosology puts more weight on the cardiovascular manifestations of the disease. We anticipate that the new nosology can delay a definitive diagnosis of MFS but decreases the risk of premature or mis-diagnosis and facilitates discussion of risk and management guidelines.

C16.2 Acquired uniparental isodisomy as a common somatic 2nd-hit explains multifocality of glomuvenous malformations

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Inherited vascular anomalies are commonly characterized by autosomal dominant inheritance with high penetrance, highly variable expressivity regarding size, number and localization of malformations, and small size of postnatal lesions. In 1994, when we identified the genomic locus linked to inherited cutaneomucosal venous malformations (VMCM), we hypothesized that the Knudson's double-hit model for retinoblastoma could be applicable to inherited vascular anomalies. Rarity of accessible resected inherited vascular malformations has hindered such studies. In 2002, we reported a somatic second-hit in one glomuvenous malformation (GVM) and in 2009 another one in VMCM. In this study, we screened 16 GVM tissues for somatic 2nd-hit mutations using DNA extracted from whole tissue or laser capture microdissected tissue, cDNA made of total tissular RNA, or whole tissular DNA for DNA microarray-based analyses. We identified a somatic 2nd-hit in 11/16 lesions (65%). Three were intragenic changes leading to altered mRNA splicing, one was a 1p21-22 deletion and seven were acquired uniparental isodisomies (aUPID) of the whole short arm of chromosome 1. Difficulty of identification, enrichment of somatic mutations by LCM and cDNA studies, and the need for pairwise copy number analysis suggests important tissular heterogeneity for the 2nd-hits. The cellular double-hits lead to localized complete glomulin loss-of-function. Thus, the inherited mutations are phenotypically recessive and need a co-existing somatic mutation, a hallmark of paradominant mode of inheritance. The data demonstrate, for the first time, that aUPID is involved in non-malignant disorders and although the 1p aUPID was specific to

GVMs, somatic UPID may have wide implications.

C16.3 'Lethal Vascular Syndrome from South India due to a Novel Mutation in Fibulin 4'

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We report a lethal disorder characterized by a distinct phenotype and arterial tortuosity in a unique cohort of sixteen infants, caused by a novel mutation in the Fibulin 4 gene.

Methods: Prospective, hospital-based study (Jan 2006 - Dec 2009)
Results: Sixteen children (9M / 7F) were identified with arterial tortuosity and a distinct phenotype, characterized by long philtrum and thin upper lip (87%), cutis laxa (53%), sagging cheeks (47%), hypertelorism (53%), and long fingers (40%). All children presented with early onset respiratory symptoms (median age 1.5 months) and belonged to unrelated families from the same geographical (South India) background with a history of third degree consanguinity in seven families. Cardiovascular features were documented by echocardiography, cardiac CT and MRI which revealed marked dilatation and tortuosity of aorta and its branches. Thirteen patients died between 36 hours -17 months of age (median 2.5 months) due to respiratory failure. Genetic studies led to identification of a novel homozygous c.608A>C (p.Asp203Ala) mutation in exon 7 of the Fibulin 4 gene in 15/16 patients. In the only patient surviving to the age of 6 years, compound heterozygosity was found for this mutation with a c.679C>T (p.Arg227Cys) mutation. Prenatal study in one couple identified an affected foetus. Haplotype analysis revealed a shared haplotype with Fibulin 4 gene, which strongly suggested common ancestry for all probands due to a relatively old founder mutation.

Conclusions: This is the first complete description of this lethal genetic disorder and illustrates that Fibulin 4 is critical to human elastogenesis and vascular integrity.

C16.4 A new locus for a syndromic form of Thoracic Aortic Aneurysms maps to chromosome 15q

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Background: Thoracic aortic aneurysms (TAA) are familial in at least 15-20% of the cases and can be classified in syndromic and non-syndromic forms. The TGF-beta signalling pathway plays a central role in the pathogenesis of both syndromic and non-syndromic TAA.

Methods and results: We present a large four-generation family with a syndromic form of TAA. Thirty family members had an extensive physical and cardiologic examination. Eleven family members were considered affected on the basis of cardiac and/or skeletal and connective tissue abnormalities. Nine patients had an aortic or other large artery aneurysm. TAA patients had a high risk of aortic dissection or rupture at an early age. Many TAA patients had additional heart malformations, including mitral valve abnormalities, persistent ductus arteriosus and pulmonary valve stenosis. Histological examination of the aorta showed medial fragmentation, disarray of elastic fibres and excess of collagen deposition.

After excluding mutations in the known syndromic TAA genes (*FBN1*, *TGFBR1*, *TGFBR2*, *COL3A1*), we performed a genome wide linkage analysis in this family using the Affymetrix 250K *Nsp* arrays. A new locus on chromosome 15q with a significant LOD score of 3.6 was identified within a critical region containing 120 genes. Sequencing of positional candidate genes is ongoing.

Conclusions: The clinical phenotype overlaps with known TAA syndromes such as Marfan syndrome, Loeys-Dietz syndrome and vascular type Ehlers-Danlos syndrome. Our data provide evidence for a new locus for a syndromic form of TAA. Identification of this novel gene will provide insight into the pathogenesis of arterial aneurysms.

C16.5 Loss of function ENPP1 mutations cause both generalized arterial calcification of infancy and autosomal recessive hypophosphatemic rickets

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The analysis of rare genetic disorders affecting phosphate homeostasis led to the identification of several proteins that are essential for the regulation of phosphate homeostasis, for example fibroblast growth factor 23 (FGF23) a phosphaturic factor, which inhibits phosphate reabsorption and 1,25-dihydroxyvitamin D synthesis in the proximal renal tubules. Cases of hypophosphatemia remain, including familial and consanguineous ones, which do not show mutations in any of the three known genes - *PHEX* (XLH [MIM 307800]), *FGF23* (ADHR [MIM 193100]) and *DMP1* (ARHP [MIM 241520]) which, if mutated, causes hypophosphatemic rickets. Here, we present three different homozygous, presumable loss of function mutations in a further gene, *ENPP1* (ectonucleotide pyrophosphatase/phosphodiesterase), in members of four families affected with hypophosphatemic rickets. Intact plasma levels of FGF23 were clearly elevated in one of five affected individuals, providing a possible explanation for the phosphaturia and inappropriately normal 1,25-dihydroxyvitamin D levels. Surprisingly, *ENPP1* loss of function mutations have previously been described in generalized arterial calcification of infancy (GACI [MIM 208000]), a severe autosomal recessive disorder with a hypermineralizing phenotype, suggesting an as yet elusive mechanism which balances arterial calcification with bone mineralization. With the identification of *ENPP1* mutations as the cause of hypophosphatemia, we added a further component to the growing list of genes involved in the regulation of phosphate homeostasis.

C16.6 Epigenotype-phenotype correlations in Silver-Russell syndrome

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Silver-Russell syndrome (SRS) is characterised by intrauterine growth restriction, poor postnatal growth, relative macrocephaly, triangular face and asymmetry. Maternal uniparental disomy (mUPD) of chromosome 7 and, hypomethylation of the imprinting control region (ICR)1 on chromosome 11p15 are found in around 5-10% and up to 60% of patients with SRS, respectively. As many clinical features of SRS are non-specific and may change with time, the diagnosis remains difficult. Studies of patients in whom the molecular diagnosis is confirmed therefore provide valuable clinical information regarding the condition. We undertook a detailed, prospective study of 64 patients with mUPD7 (n=20) or ICR1 hypomethylation (n=44). The considerable overlap in clinical phenotype makes it difficult to distinguish these two molecular subgroups reliably. ICR1 hypomethylation patients were more likely to be scored as 'classical' SRS. Asymmetry, fifth finger clinodactyly and congenital anomalies were more commonly seen with ICR1 hypomethylation, whereas learning difficulties and referral for speech therapy were more likely with mUPD7. No correlation was found between clinical severity and level of ICR1 hypomethylation. Use of assisted reproductive technology in association with ICR1 hypomethylation appeared increased compared with the general population. ICR1 hypomethylation was also observed in siblings, though recurrence risk remains low in the majority of cases. Overall, a wide range in severity was observed, particularly with ICR1 hypomethylation. No clinical feature was present in all cases and even low birth weight only in 78%. We would therefore recommend a low threshold for investigation of patients with features suggestive, but not typical, of SRS.

Abstracts of ESHG Posters

P01 Genetic counseling, including Psychosocial aspects, Genetics education, Genetic services, and Public policy

P01.01 Misunderstanding and Misuse of Genetics Counseling at the Expenses of Medical Genetics Rules and Basic Human Rights

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Genetic counseling began with the Eugenics movement in Europe and USA around 1910 leading to very destructive inhuman negative effects on health and dignity of humankind. Fortunately the misuse of Eugenics was officially abandoned in 1944. Though it persists in many developed and developing countries up to today in a rather hidden manner. Clinical genetic counseling based on DIGNITY and EQUALITY of mankind was introduced by Reed in 1944. In Iran and the Islamic countries Genetic counseling was introduced in 1968 by the first author on the same basic principles taught by his graduate mentor professors Yamada, Fuhrmann, Vogel and Reed. These Principles give consideration to ALL of the Humanitarian, Ethical, Moral, Religious and Medical issues concerning the clients and their society. On these principles, since 1968 in Iran the first author have provided a genetic counseling service to over 18500 couples seeking academic advice concerning consanguineous marriage, their risk assessments and proper medical advices.

Since 1980 due to higher demand for counseling services some unauthorized and un- or inadequately educated MD or PhD holders have started genetic counseling with improper advices or test recommendations in many occasions. These inappropriate or misused issues are NO Marriage, NO Child for many of carriers of monogenic diseases and clinically unjustified Karyotyping of quite normal and healthy young couples and forecasting all of the genetics abnormalities of conception from couples karyotypes. This last issue unfortunately is taken by the lay people as a MUST test for avoiding genetic diseases in their offspring. False positive and negative hopes as well as spending a very time consuming, expensive and inappropriate practice is among these malpractices of genetic counseling in Iran and many developing countries.

We feel it is time for WHO and international medical genetic congresses to address this very humanitarian and academic issue and draw a very precise nomenclature for the practice of Genetic Counseling all over the world. Also the very scientific fact of presence of a general 5-7% chance of having an abnormal child for every healthy, normal and unrelated young couple without any clinical indications of a genetic abnormality is a fix and fusible fact cannot be avoided through couple testing. This is the price of nature for unknown biological task of continuing generations of mankind.

P01.02** The use of stored tissue samples from children for genetic research. Ethical issues.

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Collections of biological samples have a great potential for genetic research. Much has been written in the last decade on the ethical aspects of the use of such samples from adults, but the ethical issues surrounding the use of stored tissue samples from minors continue to cause controversy. This was made clear this summer by the publication of a policy forum in *Science*. During the last three years we have conducted theoretical and empirical research on this topic, and will present the conclusions of this research in this talk. We discovered three main areas of interest. First, the requirement that research on vulnerable populations such as children should pose no more than minimal risk is problematic for biobank research, as it is as of yet unclear what risk is entailed in such research. We propose an approach of 'burden and benefit' rather than one of minimal risk to decide on the conditions for participation of children. Second, we reflected on the scope of parental consent. Should parents be allowed to give broad consent for any type of research and for an unlimited amount of time? If and when should the children themselves be involved? We

propose a solution that is both respectful of the child's preferences and that poses not too much administrative overhead for the researchers. Third, we discuss the issue of returning individual research results and incidental research findings for young participants. We argue that such results, when minors are concerned, deserve special attention in guidelines and recommendations.

P01.03 The scope of prenatal diagnostics: broad or narrow? Ethical reflections.

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Various new techniques of rapid aneuploidy diagnosis (RAD) challenge the tenability of karyotyping as the gold standard of prenatal diagnosis for pregnancies at 'increased risk'. RAD, targeted at a few chromosomal abnormalities, generates less so-called unexpected findings. This would have the advantage of simplifying counselling, alleviating the burden of choice and preventing unnecessary abortions. Furthermore, RAD would allow mending the present rift between a narrow entrance gate to prenatal screening (risk-assessment for aneuploidy of chromosomes 21, 18, 13) and the much wider scope of subsequent karyotyping.

However, RAD misses some clinically relevant abnormalities and may therefore be seen as making suboptimal use of foetal material obtained through invasive and risky procedures. Moreover, it can be asked whether the advantages of having rapid and unequivocal results justify depriving pregnant women of potential useful information. Various views on possible aims of prenatal screening are relevant to this debate, although not conclusive for determining the precise scope of prenatal testing. One possible option may be to provide women with a choice between karyotyping and RAD.

The opposing perspectives are also informed by various views on the principle of autonomy: should the emphasis be on optimizing the process of decision-making or on maximizing reproductive options? The latter view may imply welcoming the use of tests with an even broader scope than karyotyping.

Our preliminary moral assessment suggests that offering RAD as a stand alone test would unjustifiable trespass on reproductive autonomy and amount to a rather restricted view on the aim of prenatal screening.

P01.04 EPMA: Predictive, Preventive & Personalised medicine as a novel strategic trend in Europe

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Predictive diagnostics is considered as the basis for targeted preventive measures and consequent development of individualized treatment approaches. Of paramount importance is the communication among professionals - medical doctors, biotechnologists, computer-scientists, healthcare providers, policy-makers, educators, who are obligatorily involved in the paradigm change from curative to predictive medicine.

The paradigm change can be achieved only by well-coordinated action towards: adequate investment in creating novel technologies, development of non- or minimally-invasive diagnostic tools, well-organised exchange and transfer of knowledge among biomedical research entities and biotech industries for production of the advanced diagnostic tools, quality assurance through the introduction of international standards for technological tools and devices, patenting and licenses, professional education in terms of the application of biotechnological high-tech in medicine, political regulations in the health-care sector: introduction of the obligatory guidelines and clear regulations for the health insurance industry to ensure patients needs are met, measures to ensure confidentiality of patient information and personal databank and distribution of relevant information among health-care professionals and users. The mission of the **European Coordinator** in this field is performed by the "European Association for Predictive, Preventive and Personalised Medicine" (EPMA). The Association is clearly structured in order to reach the possibly best coordination of the PPPM-related multifaceted activities over the whole Europe: there are National Representatives in all 27 Country-members of the European Union and the Associated-Countries (e.g. Israel, Serbia, etc).

P01.05 TIME-trial: A multicentre study on the behavioural and psychosocial effects of rapid genetic counselling and testing in newly diagnosed breast cancer patients

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Background: In 15% of high-risk families, a BRCA1 or BRCA2 gene mutation can be found. Female carriers with breast cancer have an increased risk of new primary tumours and may opt for preventive surgery. Usually, genetic counselling and DNA-testing are offered after primary treatment and take 4-6 months. However, some Dutch laboratories can generate test results within 3-6 weeks. Little is known about the effect of such rapid genetic counselling and testing (RGCT) procedures on treatment decisions and psychosocial health.

Methods: The TIME-trial is a prospective randomized study. Newly diagnosed breast cancer patients (N=255) with 10% or greater chance of having a BRCA mutation are being recruited from 12 Dutch hospitals. They are randomized on a 2:1 basis to either a RGCT-group (referral to a clinical geneticist within one week of diagnosis), or a usual care control-group. If needed, DNA-test results are made available within 3-6 weeks. The primary study outcome is choice of surgical treatment. Secondary outcomes include perceived cancer risk, cancer-related distress, health-related quality of life and decisional satisfaction. Psychosocial assessments take place at study entry and at 6 and 12 months follow-up.

Results: Patient recruitment started in November, 2008. To date, 124 women (response 82%) have been entered into the trial. Of the 80 participants in the RGCT-arm, approximately one-third has opted for rapid DNA-testing.

Conclusion: This multicenter clinical trial of RGCT is recruiting well and, based on the figures available to date, a higher percentage of women is opting for rapid genetic testing than had been anticipated.

P01.06 Introducing a patient's choice in how to learn the results of presymptomatic DNA testing for hereditary cancer

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AIM: To test whether coping after DNA testing for hereditary cancer, is similar when counselees have the choice to learn the results by letter instead of in a face to face contact.

BACKGROUND: In pre-symptomatic testing for hereditary cancers, the availability of treatment/prevention seems to mitigate the impact of the test result, as compared to Huntington's chorea. Moreover, counselees indicated they preferred to learn the test results by letter for several reasons.

METHODS: Cases referred for pre-symptomatic testing for BRCA or Lynch syndrome were randomised between 'standard care protocol' (disclosure of DNA results in a face to face contact), and 'choice protocol' (the counselee gets the choice to either learn the result in a face to face contact or to be sent the results in a letter with a subsequent appointment). Included counselees filled out questionnaires covering knowledge, coping, stress and satisfaction before the intake session (t1), 2 days after DNA test result disclosure (t2) and 6 weeks later (t3).

RESULTS: In total 244 out of 340 ascertained cases consented in the study and returned the first questionnaire: 205 BRCA1/2 and 39 Lynch syndrome. Some refrained from or postponed DNA testing after intake and were excluded. To date 48 cases got the standard protocol (20 carriers, 28 non-carriers) and 82 cases got the choice protocol (26 carriers and 56 non-carriers). Of the 82 cases in the choice protocol, 58 chose disclosure by letter and 24 chose disclosure in an appointment. The results of our analysis will be presented.

P01.07 Cardiac genetics: the first 250 clinic patients

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In February 2007, a monthly cardiac genetics clinic was commenced at the Royal Brisbane and Women's Hospital. The clinic was to ensure appropriate counselling, genetic testing and management of those with or at risk of an inherited cardiac condition. A range of conditions have been seen including; hypertrophic cardiomyopathy, familial dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, catecholaminergic polymorphic ventricular tachycardia and long QT syndrome. Rarer conditions such as Fabry disease and Danon disease have also been seen. To date 250 patients have been seen through the clinic. Because of pressure on clinic space, a number of the patients referred to the genetic service have been seen in genetics clinics at locations around the state. A number of challenges have been encountered: management of those with a mutation but no phenotype; testing of at risk children; funding for testing; waiting times with increasing numbers of cases referred. The clinic also collaborates with the forensic pathology service and coroner in the assessment of those families where there has been a sudden unexplained death in a young individual. The data from the clinic will be presented.

P01.08 Ethical aspects of Array Comparative Genomic Hybridisation (CGH) for mental retardation diagnosis and genetic counselling

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The recent rapid emergence of new technologies in genetic medicine contributes to the diagnosis and the understanding of the molecular basis of numerous diseases. The high throughput and the sensitivity of these technologies will create an unprecedented volume of information for patients, counsellors, and health care providers, raising also unique ethical challenges.

Although the applications of many of these techniques are still limited to research, Array Comparative Genomic Hybridisation (CGH) has evolved to a standard application in clinical genetics, especially in individuals with syndromic or non-syndromic mental retardation.

CGH allows a whole genome analysis at a resolution, 10-10 000 times higher than that of routine chromosome analysis by karyotyping. This method has revolutionized the present clinical practice in the detection and diagnosis of human chromosome abnormalities in mental retardation. Some ethical issues specifically challenge the transfer of these improved technologies from the research laboratory to their clinical applications. In our experience we have identified several situations asking ethical difficulties. They will be presented according to two main dimensions: uncertainty of results and incidental findings.

Ethical issues of genetic disease diagnostics and various health applications have already been addressed in numerous reports, but with the use of these screening technologies in the clinical setting, the "incidental" becomes "usual," representing new challenges for the clinician.

Specific reference documents addressing the ethical issues of genetic testing in relation to CGH are still lacking.

P01.09 Evidence-based information guides to rare chromosome disorders for families and professionals

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Purpose: To develop reliable, relevant, accurate leaflets for affected families and health professionals that fill an information gap about rare chromosome disorders.

Method: In 2003 Unique surveyed information materials published in the UK about specific rare chromosome disorders: for over 93% of members no accessible disorder-specific information was available. Unique asked families what they most wanted to know at diagnosis

and what questions remained unanswered. *Unique* prioritised 66 disorders according to frequency on its database (7122 member families at February 2010) and absence of existing information accessible to families. Information was compiled from the medical literature; from *Unique*'s database; and from detailed surveys sent to member families. Draft texts were reviewed for accuracy by *Unique*'s medical adviser and by medical and genetics professionals expert in the specific disorders. Photographically illustrated draft leaflets were vetted for content by families.

Results: Leaflets have been developed on 113 topics including numerical and structural disorders; subtelomere deletions; mosaic disorders; emerging microdeletion and microduplication syndromes and a broad range of less common diagnoses.

Discussion: Twenty-one leaflets have been translated into at least one European language. Leaflets, available to families and health professionals in print format and online at www.rarechromo.org, improve families' understanding and acceptance of a rare chromosome disorder and help diminish the acute stress and anxiety associated with diagnosis.

P01.10 Teaching the genetics of complex traits

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The primary goals of secondary and post secondary science education are to help prepare scientifically literate citizens and future scientists. Efforts to improve science education have focused on competency standards and the professional development of teachers, and recent efforts have promoted the use of high-stakes exams and benchmarks for school performance. In spite of this work, students in the United States continue to lag behind their peers in other countries. This underperformance is true for genetics and for science and math in general, and it is particularly worrisome given the accelerating need for scientists and engineers in an increasingly technology-driven economy. Unfortunately, even students in high-performing European countries, such as Finland and Slovenia, have relatively small percentages of students performing at the higher levels of proficiency.

Perhaps nowhere is the need for scientific literacy more personally meaningful and urgent than in genetics--because of its connection with medicine. Rapid changes in human genetics--in particular recent insights into the genetics of complex traits--have the potential to transform healthcare. However, citizens are ill-prepared to participate in this transformation because the curriculum is outdated and remains focused on single-gene inheritance and simplified notions of risk. One potential solution is to modernize the genetics curriculum so that it matches the science of the 21st-century. This paper outlines the problems with current genetics instruction, highlights changes in human genetics that support a curricular reorganization, and proposes a new genetics curriculum for secondary and postsecondary education.

P01.11 Molecular genetic study of polymorphic variations in genes within the cascade of reactions detoxification of xenobiotics with aromatic structure

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Development of industrial production around the world have led to a large number of chemical compounds that adversely affect human health. Toxic substances violate the metabolism, the structure of cells and tissues, resulting in abnormal reaction of the organism. The negative effect of xenobiotics depends on the activity of physical and biochemical methods of protection, including the immune system and the system of biotransformation of alien connections.

We studied DNA samples - 714 „apparently healthy“ individuals in the two groups, differing in environmental conditions of residence in the same territory, examined polymorphism of genes within the cascade of reactions detoxification of xenobiotics with aromatic structure: CYP1A1/A4889G, CYP2E1/Ins 96,EPHX1/A4156,NQO1/C609T,C465T.

A group of environmental risk revealed a significant increase in the frequency of the protective genotype D / D polymorphism Ins96 gene CYP2E1 (79,74% against 58.44%).

Comparative analysis of the frequency distribution of genotypes and alleles of polymorphism C609T gene NQO1 revealed a statistically significant difference by increasing the frequency of the protective geno-

type C/C in the group of environmental risk (68.2% versus 59.87% in the comparison group, $P = 0.0478$). For a comprehensive analysis was studied genotypes of the five polymorphism in the studied genes. Revealed a statistically significant increase of genotypes for the study of polymorphism at environmental risk (AADDAGCCCC: $\chi^2 = 9,69$, $P = 0.0027$, AGDDAACCCC: $\chi^2 = 9,47$, $P = 0.003$), may determine that a combination of data most efficient biotransformation of xenobiotics and reduces the negative impact of environmental factors and some carcinogens in the body.

P01.12 Medical and genetic counseling of families with cystic fibrosis in the Republic of Moldova

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Cystic fibrosis is an important medico-social problem for the Republic of Moldova, which is associated with low life expectancy of patients (average age - 13,5 years), the difficulties of clinical and molecular diagnostics, low economic level of the population and lack of social support for families with cystic fibrosis.

An important step in the prevention of cystic fibrosis is a medico-genetic counseling in families at high risk. We are presenting an 18-year experience of retrospective counseling of 58 families in which there were 69 children suffering from cystic fibrosis aged from 1 month to 18 years (35 boys and 34 girls).

In 56 families there were moral and ethical difficulties with improvement of the diagnosis. Most families (63.8%) lived in rural areas, 66.4% of parents had secondary education. The survey showed that 72% of parents knew that the disease has a genetic nature. The results of DNA testing showed mutations in both alleles in 14 families, mutation of one allele in 25 families and 19 families were non-informative for molecular diagnosis. The prenatal diagnosis carried out in 6 families resulted to 4 healthy children.

Of the 58 families observed in 52 families there are no children with CF, that is, the effectiveness of genetic counseling was 89.7%. Information of parents about the presence of high risk have genetic nature of CF lead to positive changes of reproductive behavior. The prenatal diagnosis in affected families can allow decrease the frequency of ill children.

P01.13 The opinion of CF patient's parents about different aspects of genetic services in Russia

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The newborn screening for CF started in Russia in 2006. The opinion of CF patient's parents to different aspects of genetic services in Russia was estimated. 68 respondents were participated in the research. Majority of parents (90 %) have learnt about hereditary character of their child disease from the pediatrician, only 81 % of them have been referred to geneticist, and 67 % have been held DNA testing. However 90% respondents have considered, that they have understood the information about repeated genetic risk for CF. However only 50% of them could correctly specify the value of recurrence risk of CF, and only 30 % of them could correctly attribute a risk category. The birth of the CF child has affected reproductive plans in 30 % of CF families, only 35% of families wanted to have more children. 80 % of CF children's parents knew about possibility of prenatal diagnostics of CF, however almost 40 % from them have learnt about it not from experts, but from the press. Prenatal diagnostics of CF was acceptable for 66% of respondents, and the abortion of a CF foetus is morally acceptable for 82% of them. Only 4 % of respondents have answered that they didn't want to terminate the CF foetus pregnancy. In 11/15 women who were pregnant after the birth of CF child, prenatal diagnostics of CF was done: 7 foetuses were healthy, 4 - with CF. All CF foetus pregnancies have been terminated.

P01.14 Psychosocial aspects of genetic paternity testing ordered by courts: does biological relatedness create a father?

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Many European societies support efforts to establish genetic parentage of children born outside marriage when no father is listed on the birth registration. This State effort is usually part of public policies to ensure that children are cared for not only financially but also with regards to education and upbringing. There are also medical reasons to establish genetic parentage, and the importance of parents in the psychological development of infants.

We discuss the benefits and/or disadvantages of genetic paternity testing from the point of view of mothers and fathers. We focus on cases ordered by Portuguese courts of law and rely on data proceeding from interviews conducted with women and men who went through the process of DNA paternity testing. Some topics of debate are: psychosocial aspects of submission to genetic paternity testing; informed consent; access to information about genetic testing procedures and communication of results; perceptions of the role of genetic relatedness in defining fatherhood.

The results suggest that there are important psychosocial impacts produced by genetic paternity testing which question the value of the legal primacy of biological relatedness in forming fatherhood and in serving the best interests of the child. We recommend better practices of informed consent, communicating the results of paternity tests and even psychological counseling to motivate mothers and fathers for the necessity of creating and continuing a relationship with the child and between the progenitors. These practices demand a more effective coordination between courts of law and laboratories and specific training of personnel.

P01.15 DOUBLE COUSIN MARRIAGE IN THE IRANIAN PROVINCE OF HORMOZGAN (2002-2009)

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Background: Double first cousins arise when siblings of one family reproduce with siblings of another family. The resulting children are related to each other through both parent's families. Double first cousins share both sets of grandparents in common and have double the degree of consanguinity than ordinary first cousins. Genetically, they are as related as half-siblings sharing 25% of their DNA. A consanguineous couple especially double cousin couple is at increased risk for both autosomal recessive disorders and several congenital malformations. Material and methods: In seven years from 2002 to 2009, we performed genetic counseling for 2400 couples. The consanguinity between 34 couples was double cousin (1.4%). Familial pedigree established and risk of genetic disorders for their children was calculated.

Results: 1428 couples (59.5%) had consanguinity and 34 couples (1.4%) were double cousins.

-13 couples (38.3%) came premarriage, 17 couples preconception (50%) and 4 couples (11.7%) during pregnancy.

-inbreeding coefficient in 15 couples (44.1%) was more than 1/8.

-18 couples (52.9%) had positive history of genetic disorders in family.

-15 couples had children and in 14 of them (93.3%) at least one child was involved with genetic disorders.

-3 couples (8.8%) were carriers of beta thalassemia and referred for prenatal diagnosis.

Conclusion: Based on high risks for genetic disorders in double cousin marriage and according to all of couples announced "if they knew about these risks, they would avoid marriage". Performing genetic counseling is recommended before all consanguineous marriages especially double cousin type.

P01.16 Management deficiencies of Duchenne Muscular Dystrophy: A reality that we can change

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The right management of Duchenne's muscular dystrophy (DMD) should include several steps: a) clinical and genetic diagnosis and counseling, b) refer to a specialist and multidisciplinary team, c) information and support for the family.

Our study was conducted for 16 Romanian boys affected by DMD and their families.

We examined how the diagnosis was made, the application of specific treatment with corticosteroids, tracking of supportive therapy protocol, involvement of a team of specialist in the disease management, genetic counseling and psychosocial support for families.

The results of this study revealed a system of management of this disease "fractured" at all levels:

- Late clinical diagnosis (4 cases), late genetic diagnosis (8 cases)
- Incomplete diagnosis - lack of immunohistochemical diagnosis and/or molecular diagnosis (6 cases)
- Wrong diagnosis (2 cases, although diagnosed with DMD, after molecular analysis it is being evaluated for a different type of dystrophy).
- Lack of therapeutic instrument provided by a multidisciplinary team of specialists, inadequate treatment (15 cases)
- Lack of genetic counseling (5 cases) and psychosocial support to families (15 cases)

From the 16 children, only one benefits by a correctly and completely management of the disease.

In the context of the emergence of the first innovative treatment (PTC 124, exon-skipping), if we want Romanian children to have access to clinical trials, it is vital to have a standard of care compatible with the European one; the management of DMD by a network of specialists can buy time for our children and stop the reappearance of the disease.

P01.17 Population-based study of dystrophin mutations in Canada

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Background: Duchenne and Becker muscular dystrophy (DBMD) are allelic disorders caused by mutations of the dystrophin gene on Xp21. This study describes the diagnostic methods and mutation frequency among the patients of the Canadian Pediatric Neuromuscular Group (CPNG).

Methods: De-identified data containing the clinical phenotypes, diagnostic methods, and mutational reports from DBMD patients followed by CPNG centers during 2000-2009 were analyzed using descriptive statistics. Patients were considered to have had complete genetic testing if all 79 exons of the dystrophin gene were examined and if this was negative, sequencing of the gene. Diagnosis solely by muscle biopsy or PCR analysis of a subset of exons was considered incomplete genetic testing.

Results: 773 DBMD patients had a confirmed diagnosis based on genetic testing (97%), muscle biopsy (2.3%), or family history (0.7%). 573/773 (74%) had complete deletion/duplication analysis of all 79 exons or gene sequencing resulting in 366 (64%) deletions, 64 (11%) duplications, and 143 (25%) point mutations. The most common point mutations were nonsense (47%), followed by frameshift (31%), splicing (15%), and missense/amino acid deletions (7%). Access to complete genetic testing was variable and ranged from 88% for patients from ON to 35% for patients from NS.

Conclusion: This is the first comprehensive report of dystrophin mutations in Canada. It highlights the need for complete genetic testing of patients with dystrophinopathy, and the necessity for collaboration among academic centers and neuromuscular disease registries to ensure that patients are receiving optimal care and are eligible for mutation-specific therapies.

P01.18 Influencing how genetics is taught in UK secondary schools: The Nowgen Schools Genomics Programme

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Current research in human genomics has great potential for sparking interest amongst secondary school students, yet it is not prominent in UK school curricula. The *Nowgen Schools Genomics Programme* aims to redress that situation; narrowing the gap between genomics research and classroom genetics.

The genetics content of UK school curricula currently concentrates on 'single-gene' genetics with few references to more recent approaches exploring the human genome. Funded by a three-year grant from The Wellcome Trust, the *Nowgen Schools Genomics Programme* brings together leading scientists, clinicians, educationalists and bioethicists to contribute to a range of approaches designed to equip young people to assess the real potential of genomics, and to make informed decisions about future healthcare. The programme will introduce genome-wide association studies, pharmacogenetics and genetic medicine to teachers and their students, and will support them to:

- explore genetic and lifestyle contributions to disease;
- consider the methodological challenges contemporary large population studies require;
- examine the social and ethical challenges to society;
- explore the potential impact of data arising from contemporary genetics.

P01.19 DNA Day:a good starting point to disseminate genetic education in high school

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DNA Day-a special day-initiated in 2003 to commemorate the completion of the Human Genome Project in April of that year, and the discovery of the DNA double helix fifty years earlier!

In 2008 The European Society of Human Genetics (ESHG) joined the American Society of Human Genetics (ASHG) in making a Europe-wide celebration of genetics and its promises and decided to expand a very successful initiative: the DNA Day Essay contest.

That contest is one of several ongoing initiatives to promote knowledge and understanding of genetics in secondary schools.

In Italy, the Dna Day contest has been a fantastic tool and occasion to propose an advanced knowledge in genetics, increasing need of an accurate education at school.

The collaboration and support between teachers, the Italian Society of Human Genetics (SIGU) and the University of Genova has carried on initiatives of lectures by geneticists in order to close high school students to research topics, facilitate and stimulate a much more wide reflection on the essays proposed by the contest.

In 2008, in Genova 400 people (students and teachers) attended the conference at the Faculty of Medicine and in 2009, more than 250 people were present at the theatre of Advanced Centre of Biotechnology.

This year, the SIGU has set up a more strict plan to promote a genetic education project:

- addressing a letter to the high school teachers
- publishing a banner on the national website
- having a national selection and awarding three Italian students at the national meeting

P01.20 Feedback of individual genetic data to research participants: a qualified disclosure?

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Last years, a debate evolved regarding the question whether individual genetic research results should be disclosed to research participants in genetic/genomic research, and if so, which data, and by whom. With genetic research results we refer to a broad category of information, including validated and non-validated, highly and poorly predictive, and more or less probabilistic genetic data generated by a medical scientific study. On the one end of the spectrum, it is argued that full

disclosure of individual genetic data to research participants is ethically imperative. On the other end of the spectrum, it is argued that no individual genetic research results should be disclosed whatsoever. An intermediate position is defended by those who opt for a 'qualified disclosure'.

Although most commentators adopted a variant of this latter position, their underlying argumentation and the conditions for disclosure varies widely. Some for example argue that individual genetic data should only be disclosed when these data are of analytic validity and clinical relevance. Others argue that the decisive factor concerns the relationship between participant and researcher; for example, the more intense this relationship, the stronger the obligation to disclose would be.

Clarity on the appropriate standard of disclosure is important in view of the changing genetic landscape, such as the current genome wide association studies, the impending whole genome sequencing-studies and the upcoming commercial activities. In this paper, we present the results of our systematic search and explore whether and when individual genetic data should be returned to research participants.

P01.21 Construction of an ethics policy for a bioinformatics European project: GEN2PHEN

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Genomic technologies including bioinformatics methods and creation of genomic databases have become increasingly important for the scientific community but have increased faster than its possible framing by an appropriate legislation. However these activities must be developed with respect to fundamental values such as dignity, privacy, autonomy.

The lack of a binding instrument regulating all aspects of these issues and the varied and disparate legal and ethical frameworks has resulted in confusion for professionals working in genomics to construct their own ethics policies. Compliance with the various international, European, and National ethical and legal instruments requires a more considered approach.

We analysed how a specific bioinformatic European project, GEN2PHEN, is dealing with this issue in the development of its ethics policy. To provide ethical oversight, analysis, and guidance for GEN2PHEN, we considered texts and existing guidelines reviews, an international consortium questionnaire survey of opinions, discussion in general assembly, consultation with relevant EU and international projects and review of recent literature.

The main issues identified were requirements for entering data into the system, managing them over time in federated databases and conditions for their accessibility. A process for checking the computer tools produced for ethics compliance was elaborated. The policy takes into account the differences between various types of data, mandatory items of consent and modalities of their verification, clarification of responsibilities within and outside the project, data and database ownership issues and transparency of the process. It involves a project ethics oversight committee and specifies relations with local research ethics committees.

P01.22 Eurogene: a pan-european e-learning Service in human genetics

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The primary objective of the Eurogene project is to develop the means for accessing, sharing and reusing high quality educational resources in human genetics with multilingual support. The Eurogene portal offers access to learning resources produced by a network of professionals in the field represented by universities, hospitals and educational centres across Europe. The scope of Eurogene includes statistical, medical and molecular genetics and the content is targeted at people with different levels of previous knowledge ranging from the lay person

to the expert practitioner.

Working towards this goal Eurogene has created a genetic domain ontology and a hierarchy of terms that are first used to annotate and enrich the e-learning resources. This automatic annotation forms structure to support the suite of web based tools developed specifically for providing, finding and translating digital educational content in the field of genetics. Interactive machine translation tools allow approved users to refine, train and hence validate the genetic context multilingual dictionary.

The Eurogene portal exploits technology which makes it easy to navigate and explore the available articles, images and videos. Educators can thus move toward "blended learning" in an interactive and natural way, while students are given the opportunity to finally become "actors" in the educational process. We will demonstrate the main features of the tools offered by the Portal. This will include viewing recent sample content, illustration of the annotation process and machine translation options, and construction of learning packages.

EUROGENE is a 36 month project supported by the EC's e-Content Plus program.

P01.23 Detection of *FMR1* CGG repeat expansions from genomic DNA and single cells - a direct triplet-primed PCR approach

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Fragile X syndrome (FXS) is the most common cause of heritable mental retardation. It is mostly caused by the hyperexpansion and hypermethylation of CGG repeat expansions in the 5' untranslated region of *FMR1*, leading to gene silencing. There are 4 allelic classes of *FMR1* CGG repeats: normal (5-44 repeats), gray zone (45-54 repeats), premutation (55-200 repeats), and full mutation (>200 repeats). Full mutation alleles are associated with FXS, while unmethylated premutation alleles have been associated with premature ovarian failure (POF) in females and with late-onset fragile X-associated tremor ataxia syndrome (FXTAS) in males. Molecular diagnosis of FXS usually involves PCR across the CGG repeat to determine repeat length, supplemented by Southern blot analysis for male samples with null amplification or female samples with a single allele size by PCR. We describe a highly specific direct triplet-primed PCR (dTP-PCR) approach to detect *FMR1* premutation and full mutation expansions. Unique capillary electropherogram patterns also enable determination of repeat lengths and structures of normal, gray zone and small premutation alleles in all males and some females. Detection sensitivity is at the single cell level, paving the way for direct detection of expanded *FMR1* alleles in preimplantation embryos. For more rapid single-step screening, dTP-PCR products can be immediately subjected to melting curve analysis in the presence of SYBR Green dye to yield distinct, clearly differentiated melting peaks from normal versus expanded alleles, demonstrating the utility of this homogeneous assay as a rapid first-line screen for FXS, POF and FXTAS.

P01.24 Linking a genealogical database and a Cancer Registry to generate comprehensive pedigrees for cancer risk assessment.

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Genetic cancer counselling is generally based the counselee's pedigree, i.e. information provided by the counselee and confirmed, when possible through hospital records and occasionally a cancer registry. Electronic genealogical databases allow quick construction of an extensive pedigree, encompassing individuals beyond the counselee's knowledge. The Icelandic Genetics Committee Database (IGCD) holds accurate genealogical information based on official demographic records about individuals living in Iceland since 1850. The Icelandic

Cancer Registry is a population-based databank with all individual cancer information recorded since 1955. A database-generated pedigree (DGP) can be made by linking IGCD pedigrees to records in the Cancer Registry using personal identification numbers. This results in a very comprehensive cancer family history, potentially supporting more efficient assessment of the individual's inherited cancer risk.

In Iceland, cancer genetic counselling based on DGP has been offered since 2007 and 310 counselees have used the service, the majority (97%) because of a breast cancer family history. A counselee's pedigree is constructed as well as a DGP (3-4 generations with 150-1600 individuals on each side). The DGP is not revealed at the personal level to the counselee for privacy reasons. Risk assessment for cancer, including possible indication for molecular testing, is based on both the counselee's pedigree and the DGP. We are not aware of this approach resulting in any problems or privacy concerns.

The DGP and the counselee's pedigree need to be compared including cost efficiency and predictive power. If advantages are demonstrated, other communities could address legal issues and generate their genealogical databases.

P01.25 Participation of nurse in genetic counseling; present status in Japan

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⁴Department of Neurology, Tokai University School of Medicine, Isehara, Japan. Objective: Since 2005, Tokai University had started a Master course of "genetic nursing" in School of Health Science, which is only one course in Japan. To improve this condition, we are continuously emphasizing several merits in participation of genetic nurses in genetic counseling clinics. In this study we tried to elucidate the present status of participation of nurse(s) in clinical genetic counseling in Japan, as well as the expected roles of nurse(s) from other medical professionals in genetic counseling team.

Method: Questionnaires were mailed on January 2009 to 129 facilities, which are registered as genetic counseling clinics in Japanese Society of Obstetrician and Gynecologist. We focus in the prenatal diagnosis, which is the most common subject in the a genetic counseling clinic, Results: Answers were retrieved from medical doctor, nurse, and psychologist, where the response rate was highest (36.2%) from doctors. Nurses are incorporated in the team on 37.0% of responded facilities, where their role is "guidance on the patient's daily activity" before and after the amniocentesis. On the other hand, they give crucial information about the procedure in fewer occasions. Medical doctors expect nurses in a future to collect the information for counseling, to make a precise pedigree, and to shape up the client's will.

Conclusion: Comparing our previous research result, more nurses participate in genetic counseling than double of those on 2000. However, the distribution imbalance between the facilities becomes more prominent than the past.

P01.26 Developments in Genetic Counselling: a European Perspective

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The European Network of Genetic Nurses and Counsellors was formed in 2008 and now exists under the auspices of the Ad Hoc Genetic Nurse and Counsellor Accreditation Committee of the European Society of Human Genetics. Currently there are almost a hundred members. There is wide variation in the profession across Europe, with many registered practitioners in some countries, and few or none in others. In the interests of patient safety, common minimum standards are required. The aims of the network are to ensure that professionals using the title 'genetic counsellor' are competent to practice. Through a series of workshops and consultation with the membership, guidance for the future of the profession has been agreed.

The role of the genetic counsellor includes: identifying the needs of the family, collecting and interpreting genetic information relevant to genetic counselling, making a genetic risk assessment and communicating information about the risk and options available to the family. It

is envisaged that by 2020 the standard training and education for a genetic counsellor will be the Master degree in genetic counselling. The content of these educational and training programmes must include: human genetics, medical genetics, counselling skills and clinical experience. Genetic counsellors will be expected to practice according to the Code of Professional Practice for genetic counsellors in Europe. Further details will be presented and work is now required to consult widely with those from other professional disciplines to ensure that best practice in genetic counselling is adopted in Europe.

P01.27 Ethical Aspects of Genetic Testing in Russia

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Research Centre for Medical Genetics of RAMS, Moscow, Russian Federation. Studies of ethical problems concerning the application of genomic technologies in public health care in Russia commenced in the late 1980s in connection with the works according to the Russian programme „Human Genome“. These studies showed that the majority of Russian medical geneticists were inclined to be too directive, especially in cases of genetic counselling concerning prenatal detection of foetal pathology. A substantial part of the specialists (approximately 10 - 20 %) were inclined to in some way or another exert pressure on the woman's procreation choices (i.e., trying to impose or convey a feeling of an incorrect nature of the actions being taken, to persuade a woman not to have children). Russian specialists turned out rather reluctant to reliably protect the patient's confidentiality. For example, a considerable part of geneticists in Russia (about 60 %) strongly believed that bank-stored DNA samples should be readily available to the blood relatives of the patient without prior consent of the latter. Hence, the substantial part of Russian geneticists were inclined to both paternalistic decisions and directiveness. This was largely predetermined by insufficient legal regulation of the genetic service's activity in Russia, the peculiarities of the system of organization of the genetic service, poor availability and accessibility of the facilities engaged in providing proper treatment of hereditary diseases, as well as medical and social rehabilitation. Based on the obtained findings, studying of ethical issues in medical genetics was included into the professional training programmes.

P01.28 Genetic Counselling in Forensic Medicine

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Background: Genetic counselling is becoming more general medical service these days. In our country, this situation is same as other countries and Genetic Counsellor and Clinical Geneticist are increasing recently. However they are not enough for covering all patients still now. In fact there is no report of collaboration between Clinical Genetics and Forensic Medicine at present.

Aim: To develop good relationship with Forensic Medicine and provide fair genetic support after sudden death of family member because of genetic disease.

Methods: To elucidate the better situation of genetic counselling, we examine our previous two cases and estimate needs of genetic support from national and our University's sudden death statistics.

Result: In our previous medical service system, it was difficult to make any contact with genetic staffs from the family members because forensic medicine is not a clinical department. However, at our University Hospital, we had 41(7.2%) sudden death cases related to genetic condition out of 569 sudden death in total (2005-2008). Most cases were male between 20's - 40's and had genetic heart condition (hypertrophic cardiomegaly, long QT syndrome, familial hypercholesterolemia, Bur-gada syndrome, etc), Marfan syndrome and Polycystic Kidney.

Conclusion: For the bereaved family, we could provide more efficient medical service not only physically but also mentally. It will make the family members to see doctors for regular check up and avoid another future sudden death. It is very important that the collaboration between Genetics and Forensic medicine.

P01.29 MPAG - first steps of a competences-centred programme for genetic counsellors in Portugal

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Genetic counselling is an essential area in healthcare. Educational programmes for non-physicians exist since 1969, in the USA. In Europe, the first started in 1992, in the UK (Manchester and Cardiff). In the last 5 years, new master courses began in Europe (Bergen, Marseille, Genoa, Barcelona, Groningen and Uppsala).

In 2009, we initiated a two-year full-time master programme (120 ECTS). It is limited to 5-6 students (nurses, clinical psychologists), with some previous clinical experience. Its main objective is to train professional counsellors, to join multidisciplinary teams at medical genetic services and consultations.

A structured curriculum was based on the ESHG core-competences for genetic counsellors. The course consists mainly of small-group tutorials and has a large practical component. Educational areas are (1) principles and practice of genetic counselling; (2) human and medical genetics; (3) communication skills, clinical psychology, mental health, and psychosocial genetics; (4) public health, community genetics, organization of services, health policy and health economy; (5) quantitative and qualitative methods and research methodologies; and (6) bioethics and medical ethics. Nuclear disciplines in these areas are required, as are clinical rotations (prenatal diagnosis, paediatrics, neurological disorders, cancer genetics, clinical psychology); a large range of optional disciplines and clinical rotations are also offered as part of the first curricular year. The second year is fully in training at a medical genetics unit, and includes a research seminar.

We expect this to harmonize with other European programmes and help moving toward recognition of the profession of genetic counsellors in Portugal and in Europe.

P01.30 Genetic counsellors and predictive medicine : The profession and her evolution in France

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The professional master of Human Pathology, „Genetic Counsellor and Predictive Medicine“ welcomed the first students of this speciality in France in the academic year 2004-2005. Today, 72 graduates have been trained to practise this new profession of health, which is governed by a law, in different genetics services throughout France and abroad.

Using data from the French Association of Genetic Counsellors, we present this phase in the evolution of the dynamic and multidisciplinary profession of genetic counselling. This course has a vital role in the recruitment and training of these practitioners.

The diversity of skills acquired through the course include, for examples, to establish relationship and to clarify patient's concerns and expectations, to make appropriate and accurate genetic risk assessment, to work with a multidisciplinary health team, to integrate ethical and legal aspects, to use information systems, and to contribute to the research and education.

Genetic counsellors have been integrated into multidisciplinary centers for prenatal diagnosis and are involved in consultations and the predictive diagnosis of assisted reproduction. Graduates are also working in different services like medical genetics and oncology, cardiology and neurology.

We also assist in the efforts made by the french genetic counsellors to integrate national professionals in genetics into the FFGH (French Federation of Human Genetics) and the Group of Genetics and Cancer. French genetic counsellors are also participating with the ESHG and the European Network of Genetic Nurses and Counsellors to write the professional and educational standards for genetic counsellors in Europe.

P01.31 Reporting on genetic disorders and syndromes in Cyprus

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Cyprus is a small island, of 867,600 inhabitants, in the eastern Mediterranean. First human activity in 10,000 BC was followed by a Greek settlement (1400BC-1050 BC) and many invaders added to the genetic pool.

This study reports on the diversity of diagnosis established for 3400 individuals in the years 1995-2009. A registry reports on medical and demographic data. The population, initially mainly Greek-Cypriots, few Turkish Cypriots, Maronites, and Armenians, gradually became of mixed descent or more multiethnic. Referrals included all common indications.

1007 patients were diagnosed. Monogenic disorders represented the largest category while chromosomal aberrations were less frequent. Neurofibromatosis type 1 and Trisomy 21 were the commonest accordingly. 96 patients were reported with multifactorial congenital anomalies and exposures accounted for 24 patients. 986 individuals were referred for genetic counseling for various indications (except Thalassemia which is managed by the National Thalassemia center).

Presymptomatic genetic counseling and testing was offered to 290 individuals. Several rare and very rare syndromes were recognized.

Discussion: Founders' effect is evident in several communities and geographical areas. Congenital anomalies are under-represented probably due to the inconsistency in reporting and a high uptake of prenatal surveillance leading several affected pregnancies to termination. Genetic counselling initially was not appreciated but is now increasingly demanded.

In conclusion, we are reporting on the evolution of clinical genetics services and the epidemiology of genetic disorders in Cyprus in the years 1995-2009.

P01.32 The genetic testing offer in Europe: the need for cross-border testing

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Genetic tests are now offered internationally, through both public and private sector genetic testing services. Physicians prescribing these tests and biologists receiving the samples need to know which tests are available, where they are performed and whether identified laboratories meet quality standards. To fulfill this need, www.orpha.net was launched thirteen years ago to set up a database of clinical laboratories in the field of rare diseases. Data was collected in 1 country in 1997, 15 in 2003, 26 in 2006 and 38 in 2010, with resources from the EC DG Public Health. In collaboration with the EuroGentest Network of Excellence, information on quality management has been added to the Orphanet database over the past four years. Information on genetic testing in Orphanet can be searched by disease name or gene (symbol or name in English) as well as by laboratory or professional. The information provided on laboratories includes data on quality management. Currently, 956 laboratories offering tests for 1,559 genes are registered. The test offer differs greatly from one large country to another: Germany (1,141 genes), France (874 genes), Italy (625 genes), Spain (582 genes), UK (414 genes). Medium and small-sized countries' test offer ranges from 1 to 233 genes. This situation explains the large cross-border flow of specimens, highlighting the need to provide access to services in other countries when necessary, especially for very rare diseases. According to available data, only testing for Cystic fibrosis is provided by every country. The distribution of this test offer will be presented.

P01.33 Achieving change in clinical management: results from a genetics education intervention

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While there is a continual emphasis on the need to educate non-genetic specialists in genetics, does education actually make any difference to the clinical management of patients? To answer this question, the UK National Genetics Education and Development Centre developed

a half-day workshop for general practitioner (GP) trainers involved in supporting the genetic component of the national curriculum for general practice training. Instead of delivering the content as a series of didactic lectures, the workshops were designed so that content was provided using interactive case studies. This allowed GPs to draw on their own clinical experience, and highlighted where genetics was relevant in primary care practice. Sessions were evaluated using a multi-staged approach, including evaluation forms immediately after the workshop (n=51), and follow-up questionnaires (n=17) and interviews (n=4) several months afterwards. Findings showed that the structure and delivery of the workshop served as a model for good teaching practice, with participants using similar interactive case studies in their own teaching sessions. In addition, participants reported how using the clinical scenarios in the workshops allowed reflection on their own practice, which has resulted in GPs changing their clinical management. All GPs trainers (n=17) who returned the follow-up questionnaire reporting changes in their clinical practice such as: changes to referral patterns; use of family history within consultations and improved communication with patients about genetic issues. The National Genetics Education and Development Centre is now offering this course to GP trainers throughout the UK.

P01.34 Large normal („intermediate“) [27-35 CAGs] and reduced penetrance [36-39 CAGs] alleles are not a rare event in Huntington disease (HD)

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Four classes of alleles exist in HD, according to (CAG)n size; nevertheless, only two outcomes ('carrier' or 'non-carrier') are usually discussed in genetic counselling, as the possible results of presymptomatic testing (PST). Though large normal alleles may produce 'de novo' expansions, little is known about their prevalence and impact in daily practice.

We estimated the frequency of large normal (class 2) and reduced penetrance (class 3) alleles, and the frequency of genotypes carrying them, in (1) our diagnostic laboratory (1,214 samples, 350 families), (2) our genetic counselling clinic (146 testees), and (3) our general population (2,000 control chromosomes).

Large normal alleles were 6% of population control alleles, 7% of consultands for PST, and 7% of all diagnostic samples (they represented 5% of all normal HD alleles at the laboratory). Reduced-penetrance alleles were found in only one control chromosome (0.1% individuals), but in 5% of PST, and >2% of all lab samples (they were 4.3% of all expansions found, and >9% in PST).

Evidence thus showed that large normal alleles are relatively frequent in the general population, while reduced penetrance alleles are also not rare at the lab or the clinic. Together, these two classes were present in >10% of all consultands for PST.

The four existing classes of alleles must be addressed in pre-test counselling. If not aware of all possible test outcomes, consultands may become very distressed when learning that their results fall within a grey-zone, rather than providing a definite prognosis for themselves and/or their children (<90% cases).

P01.35 Clinical Coding of Rare and Genetic Diseases

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NGRL is participating in a clinical coding project that includes mapping the Orphanet (www.orpha.net) rare disease catalogue to three established medical terminologies, including SNOMED CT, in order to improve the representation and traceability of rare diseases in health records and systems. Orphanet medical terminology contains data on approximately 6,000 rare (largely genetic) diseases, relating clinical signs and genes to diseases, including epidemiological data, as well as classification of rare diseases. The Orphanet group are revising both their classification of rare diseases and the ICD-10 classification of rare diseases as part of this project and the development of ICD-11.

In order to enforce the consistency of Orphanet data we developed a logical (OWL) model and used automated reasoning to detect errors. For example, we identified instances where epidemiological constraints were inconsistent between a disease and its sub-categories,

implying either errors in classification or in the epidemiological data. Ensuring that the classification of diseases is correct is of great importance for producing valid mappings between different terminologies and in ensuring the utility of the terminologies.

The mapping process included several steps. The first, lexical mapping, based on syntactic comparison of disease names, provided us with a set of exact and partial matches. The second step included the quality control of these mappings by clinical experts, and the last step used structural data, such as classifications of diseases, to provide additional partial mappings. Our final goal is to revise SNOMED CT's data on rare diseases by using mappings from Orphanet to this terminology.

P01.36 From newborn screening to primary prevention of hemoglobinopathies

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Newborn screening for sickle cell disease (SCD) started in the Netherlands upon approval of the national health council. This was after growing numbers of patients were born in the Netherlands, due to increased immigration from endemic regions, their endogamous partner choice, and their relative high number of consanguine marriages. The first year of screening revealed a hemoglobinopathy in 64 children (0.35‰) 41 of which had SCD and 806 (4.2‰) was a HbS carrier. The detection of carriers of sickle cell offers parents the chance to prevent a possibly affected child in the next pregnancy. After indentifying a carrier the parents ought to be offered diagnostics and when both are carriers the couple at risk should be referred to a genetic center. Therefore, we monitored the hemoglobinopathy intakes at the genetic centers since the start of the newborn screening.

We asked all 8 clinical genetic centers in the Netherlands for their HbP counseling's and gave them a short questionnaire per case asking: reason for referral, ethnic background, diagnosis and plans with regards to family planning. We got cooperation of 7 centers that provided us with their anonymized data. Preliminary results show incidental referrals after newborn screening. Reasons for not seeing these families in the genetic centers may vary and remains unknown. One of the reasons maybe the pediatricians treating the affected children counsel these families themselves, but in a worse case scenario parents get no counseling and may be confronted with another ill child, without this being an informed choice.

P01.37 Clinical geneticists tomorrow: Are quantity & diversity of information an opportunity or a trap?

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Clinical geneticists have to interpret genetic data coming from the laboratory and to position themselves in the "information society". Their interpretative task tightly depends on 1. the nature of data provided by genetic testing technologies and 2. the knowledge of the patient. Patients can indeed access information and their own understanding of genetics is a relevant parameter in the patient/clinician communication. Through the development of new technologies - both in the laboratory and in the society - clinical geneticists will therefore have to face new kinds and new sources of information.

Rapid developments of high throughput technology (HTT) let expect that new genetic testing technologies will be implemented soon in clinical laboratories. Sequencing genes generates quantitatively abundance of data and qualitatively complex and heterogeneous information with different levels of clinical relevance. As data interpretation becomes tougher and unexpected health related information are more common, emerging technologies will obviously challenge medical practices.

At the same time as clinicians deal with growing uncertainty, new technologies of communication provide diverse sources of information to patients. Networks strengthen patients' communities, websites make genetic testing available directly to consumers and information flows literally on line. Diversity of information challenges the clinician's

speech: he is not the unique information provider and his patient has his own opinion about genetic information.

We therefore aim to discuss the potential impact of this evolution on patient/clinician relation and its incidence about clinical practice.

P01.38 The study of polymorphic variants in the serotonin receptor gene HTR2A have professional athletes.

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Important indicators of the success of sporting activity is a resistance to psychological stress, the ability to receive and processing of information, largely determined by the work of the neurotransmitter systems. One of the base genes neuromediator serotoninergic system is the serotonin receptor gene HTR2A localized on 13.q -14 - q 21 chromosome. The analysis of frequency distribution of alleles and genotypes of the serotonin receptor in the group of sportsmans. The analysis of the frequency distribution of alleles and genotypes of the serotonin receptor in the group of sportsmen. The analiz polymorphism A1438 in the gene HTR2A conducted in 250 unrelated individuals by PCR with subsequent processing amplifikons appropriate restriction endonuclease. Of these, 150 people who are not involved in sports, and 100 human professional athletes. Found a significant increase in the frequency of allele G (P = 0.01) and genotype GG (P = 0.005) in the group of athletes on the background of reducing the frequency of allele A (P = 0.002) and AA genotype (P = 0.005) in the comparison group. Allele G polymorphic locus HTR2A in gene A1438G have may a positive potential for sports activities. This work was partially funded grant from the Ministry of Education of Russia „Thematic Plan for 2008-2010“.

P01.39 Psychological well being and quality of life in presymptomatic Huntington's Disease gene carriers.

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Purpose: The aim of the study is to describe some preliminary data about psychological well being and quality of life in presymthomatic Huntington's Disease (HD) gene carriers after 6-12 months from DNA testing. The study is part of a larger, long-term research and has only a descriptive purpose because of the small number of the sample.

Methods: From May to December 2009, at the Institute of Genetics of Hospital/University "S. Maria della Misericordia" of Udine (Italy), we evaluated 10 presymthomatic subjects resulted carriers of HD gene. The instruments used are: 1) a semi-structured interview; 2) the Psychological General Well-being Index (PWBGI); 3) the Short Form 36 Health Survey Questionnaire (SF-36).

Results: Until now we assessed 10 patients (M:4, F: 6; mean age: 50,20, range age:28-75). The means and standard deviations of the PWBGI scales are: Anxiety 15,60 (4,90), Depression 10,80 (2,57), Well-being 10,40 (3,56), Self control 9,70 (3,53), Global health 9,60 (4,55), Vitality 10,20 (4,18). The means and standard deviations of the SF-36 scales are: Physical functioning 70 (30,83), Role-Physical 47,5 (50,62), Bodily pain 78,6 (30,13), General health 53,22 (31,66), Vitality 51,11 (25,34), Social functioning 72,50 (28,75), Role-emotional 60,00 (51,64), Mental health 62,22 (18,98). Our mean scores found to be lower than normative scores.

Conclusions: Our preliminary descriptive results indicate a critical condition in the psychological well being and the quality of life of the presympotamic HD gene carriers and suggest the importance of a specific psychological intervention. Therefore, we consider necessary to continue this study with a larger sample.

P01.40 An analysis of the impact of illness representation in predicting distress in breast cancer patients; implications for genetic counselling

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The role played by psychological, social and cultural factors in health and illness related behavioral and emotional responses is no longer breaking news. Individual differences such as various cognitions have been shown to contribute in variability in distress when confronted with

stressful events or certain illnesses. Still, there is considerable lack of empirical data concerning the impact that illness representation has in predicting distress for patients diagnosed with breast cancer. The purpose of this mixed methods study was to investigate the interrelations between illness representation and distress levels in breast cancer patients. The illness representation of 30 breast cancer patients was evaluated with semi-structured interviews; distressed was assessed using the Beck Depression Inventory, State Trait Anxiety Inventory and the Profile of Mood States. Results revealed no significant differences between levels of distress for different representations of illness ($p>0.05$). Implications for genetic counselling are discussed.

P01.41 Genetic causes of intellectual disability

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This paper reviews several genetic syndromes that are associated with intellectual disability (ID). ID is a condition characterized by significantly sub-average intellectual functioning (often expressed as I.Q. < 70 to 75) combined with limitations of > 2 of the following: communication, self-direction, social skills, self-care, use of community resources, and maintenance of personal safety. Between 1 % and 3 % of a population have an intellectual disability. Management consists of education, family counseling, and social support.

We want to present 5 cases: Cri du chat syndrome, Down syndrome, Turner syndrome, Coffin-Lowry syndrome and Sotos syndrome. All these cases have in common: delayed intellectual development, immature behavior, limited self-care skills, but genetic causes are different. All children with an intellectual disability (mental retardation) or global developmental delay should have a comprehensive evaluation to establish the etiology of the disability.

Family support and counseling are crucial. As soon as ID is confirmed or strongly suspected, the parents should be informed and given ample time to discuss causes, effects, prognosis, genetic recurrence risk, education and training of the child, and the importance of balancing known prognostic risks against negative self-fulfilling prophecies in which diminished expectations result in poor functional outcomes later in life.

A diagnosis also avoids unnecessary testing and can lead to opportunities for improved health and functional outcomes. Finally, early comprehensive prenatal care and preventive measures prior to and during pregnancy increase a woman's chances of preventing intellectual disability.

P01.42** Public interest in depression-risk genotyping in a large national sample.

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Introduction: Despite international concern about unregulated predictive genetic testing, there is surprisingly little data on both the determinants of community interest in such testing and its psychosocial impacts.

Methods: A large population-based public survey with community dwelling adults ($N=1046$) ascertained through random digit dialling. Attitudes were assessed via structured interviews.

Results: The study found strong interest in predictive genetic testing for a reported susceptibility to major depression. Once perceived benefits and disadvantages of such testing had been considered, there was significantly greater interest in seeking such a test through a health care provider (62%) compared to direct-to-consumer (40%) ($p<0.001$). Personal history of mental illness ($OR= 2.53$, $p<0.001$); self-estimation of being at higher than average risk for depression ($OR = 1.84$, $p<0.001$); belief that evidence of genetic component for a mental illness would increase rather than decrease stigma ($OR=1.51$, $p<0.001$); sexual abuse as a causal attribution for depression ($OR = 1.24$, $p <0.001$) and endorsement of perceived benefits of genetic testing ($OR=3.18$, $p<0.001$) significantly predicted interest in having such a test.

Conclusions: Despite finding attitudes that genetic links to mental illness would increase rather than decrease stigma, we found strong

community acceptance of depression-risk genotyping, even though a predisposition to depression may only manifest upon exposure to stressful life events. Our results suggest there will be a strong demand for predictive genetic testing for complex multifactorial disorders.

P01.43 Genetic counselling and testing in inherited monogenic cardiac disorders : interviews with patients and their families

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Experience with genetic counselling and testing for monogenic cardiac disorders is limited. What has been learned from dealing with other conditions like Huntington's disease or familial cancer disorders may be applicable in these families too but the context of inherited cardiac conditions present very particular problems, especially sudden cardiac death in young adults, restrictions placed on exercise and uncertainty regarding the prognosis. In addition to the advantages and disadvantages of diagnostic testing from a clinical perspective, there are issues of privacy or disclosure and coping within the family with the risk of transmission and the possible psychological consequences. One issue that requires particular attention is how families experience and cope with being told that several of them may die at any moment. Problems with the transmission of such information take different forms depending upon whether the information is to be passed "vertically" to a child or "horizontally" to a sibling or other relative and also depending upon the availability of preventive and/or therapeutic strategies. These issues were discussed in a small number of interviews with patients and/or their families, who have experienced cardiac death due to an inherited cardiomyopathy in a young adult first degree relative. We present their comments about genetic counselling and genetic testing. It clearly emerges that genetic counselling is a key component in the overall management of families affected with inherited monogenic cardiac disorders and that more research in the personal experience of these families is necessary to improve medical management and support.

P01.44 Genetic counselling and testing in Cardiomyopathies

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The recent and major breakthrough in the molecular genetics of Cardiomyopathies has created a new understanding of these diseases, and also new expectations regarding the applications in clinical practice. A new task for Cardiologists and geneticists in the cardiovascular settings is therefore to integrate this new knowledge in order to improve the management of the patients and their families. Beyond the traditional goal of genetic counselling, defined as "a communication process which deals with the human and psychological problems associated with the occurrence or the risk of occurrence of a genetic disorder in a family", the main goals are (i) to inform the patients and family about the genetic aspects of the disease, including the risk of transmitting the disease within the family; (ii) organise appropriate cardiac screening and follow-up of the relatives; (iii) discuss genetic testing, which may improve the medical management in various situations.

By showing a clinical and diagnostic work-up of a family with a compound heterozygosity in the MYBPC3 gene, these objectives are achieved taken into account the specific issues related to a genetic disease, such as the psychological, social, professional, ethical and legal implications. Genetic counselling and genetic testing should therefore be performed by trained health care providers and usually through a close collaboration between different and complimentary specialties.

P01.45 The neonatal screening programme in Leningrad province: 2009

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Academy for Postgraduate Studies, St.Petersburg, Russian Federation. Genetic neonatal screening is an important public health and clinical activity. In Leningrad province the neonatal screening programme was expanded from 2 to 5 disorders for which screening met the Wilson and Jungner criteria, especially regarding treatability. In 2009 Labora-

tory of molecular genetics and cytogenetics (LMGC) as a unit of the District Children Hospital realized neonatal screening for phenylketonuria (PKU), galactosemia (Gal), congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH), cystic fibrosis (CF). Guthrie blanks spotted with newborn's blood (12800 infants) were analyzed with automatic fluorescent methods and fluoroimmunoassay. The "PKU-neoscreen" reagents (ZAO "Immunoscreen" Moscow, Russia) were used in PKU screening. Screening for Gal was realized with Neonatal Total Galactose kit (PerkinElmer.Inc). The Delfia® Neonatal hTSH assay was used in CH screening. A risk group for CAH was formed according to the data of the Delfia® Neonatal 17- α -OH-progesterone kit. CF screening was based on the Delfia® Neonatal IRT assay. Two infants had constantly high levels of Phe and were found to be PKU infants. 30 babies were found to be a risk group of CH. 17 infants formed a risk group of CAH. Nobody was shown to be included in risk lists of Gal and CF. Clinical geneticists of LMGC confirm hereditary disorders, carry out long-term outpatient care, dietary management and genetic counselling

P01.46 Newborn hearing screening

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Background: newborn hearing screening has been instituted in many countries since the identification of congenital hearing loss in the neonate period, followed by intervention within the first few months, is critical for limited communication, low school achievements and life-long dependency. In the absence of newborn screening, the hearing loss might not be noticed by parents until the child begins to have difficulties at speaking and learning at a later age. Approximately 90% of children with deafness are born to hearing parents. Most cases with non-syndromic deafness inherited the disorder in an autosomal recessive manner.

Aims of the study: identification of congenital hearing loss in children before three months of age; detection of risk factors known to be associated with congenital hearing loss.

Methods and Subjects: Otoacoustic emissions (OAEs) and auditory brainstem response (ABR) were performed for 4303 newborns. Physical examinations and family history were used to get information about hereditary / syndromic hearing loss.

Results: 23 newborns did not pass the OAEs and ABR. All of them were referred to audiologic evaluation to confirm if the hearing loss is present. Physical examination revealed no other findings associated with a syndrome. Four newborns with hearing parents were found to have sensorineural hearing loss. They were referred to genetic counseling and testing for the etiologic diagnosis.

Conclusion: screening newborns for hearing loss is highly accurate and leads to earlier identification and treatment of infants with hearing loss. All newborns identified with congenital hearing loss require a comprehensive genetic evaluation.

P01.47 Pre-implantation Genetic Diagnosis in Saudi Arabia: Parents Experience and Attitudes

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Background preimplantation genetic diagnosis (PGD) has been proposed as a valuable alternative to prenatal diagnosis (PND) to select genetically 'normal' human embryos and transfer them to the uterus of a woman. This study evaluates a range of social and moral concerns of Saudi couples with and without experience of the IVF procedure might have about the various procedures available.

Methods A total of 184 subjects attending the King Faisal Specialist Hospital and Research Centre in Riyadh were interviewed using a semi-structured questionnaire. 87 of the subjects have complete at least one cycle of the in vitro fertilization (IVF) procedure.

Results Forty-nine (100%) of the oncology group and forty-three (90%) of the ENT group were personally accept PGD technology. All the oncology and ENT group who would personally consider PGD were willing for others to be offered the procedure. Specific concerns about PGD related to the IVF procedure, waiting for the pregnancy results and egg collection were the most commonly mentioned concerns. All

the groups of the subjects (100%) agreed that the destruction of an embryo prior to implantation less wrong than destruction of the fetus inside the uterus in comparison to only 30% of the PGD group. Views were more mixed for the other (IVF) procedure.

Conclusion The outcomes of this study demonstrate that PGD might be considered as a valid alternative to prenatal diagnosis. However, couples referred for PGD must be selected and counselled appropriately, considering the complexity of the treatment and the relatively low take-home baby rate.

P01.48 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 structure of the public attitude toward the genome research by the latent class analysis. The nationwide surveys about the attitude toward the genome research were conducted in 2005 and 2009 in Japan. The participants 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 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. They were able to be named "Group of aggressive promotion" (40.8%), "Group of passive support" (20.2%), "Group not making judgment" (18.5), "Group making prudent judgment" (16.5%), and "Group not interested in genome". The results in 2009 were almost the same as that in 2005. It is possible to forecast to which cluster to belong according to respondent's attribute, and we can forecast the reaction to other questions by using a cluster oppositely. For examples, "Group of aggressive promotion" is the layer of a high academic background, and is positive to donate their blood for the genome research, "Group making prudent judgment" is high academic background persons as same as "Group of aggressive promotion", and is interesting in the science and technology, but is negative to the blood donation for the research.

P01.49 Cross-disease support group

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Background: Rare diseases are individually uncommon (affecting fewer than 1 in 2000) but collectively affect approximately 1 in 10. Parents of children with very rare and undiagnosed diseases often feel hopeless and alone.

Hypothesis: Support addressing the needs and isolation of individuals and families with very rare and undiagnosed diseases is possible within a diverse community.

Results: To test this hypothesis, we profiled a series of individuals and families with very rare and undiagnosed diseases. Shared features that we identified included sense of isolation, need for story-sharing and desire for more information. Distinguishing features that we identified included parents experiencing greater anxiety with new compared to more established diagnoses and having an undiagnosed compared to a known condition. The parents identified common themes of basic medical, educational, social and advocacy needs. Based on these commonalities, we formed a trial support group composed of 19 families with 18 diagnoses. Over the course of 2 years, our observations are that it is possible to provide cross-disease support on issues relevant to a variety of disorders.

Conclusion: Cross-disease support groups are very effective for addressing the shared medical, educational, social, advocacy, and research needs of the community with very rare and undiagnosed diseases. Our model allows universal establishment of such local support groups

P01.50 WHO International Classification of Diseases (ICD)**Revision Process: State of Art for genetic diseases****S. Aymé, B. Bellet, A. Rath;***Orphanet - INSERM SC11, Paris, France.*

WHO has established various Topic Advisory Groups to serve as planning and coordinating advisory bodies in the update and revision process for specific areas of the ICD. A TAG for rare diseases (including all genetic diseases) was established in April 2007 as rare diseases should now be traceable in mortality and morbidity information systems. The production of the basic information needed to establish an Alpha draft of the classification of rare diseases has been assigned to Orphanet. The Orphanet database includes over 6,000 distinct phenotypes which are classified according to published classifications. These classification systems are mainly based on scientific grounds (aetiology and mechanism). Orphanet has developed a complementary, strictly clinical in-house classification to meet clinicians' needs. Available on Orphanet's website, this classification serves to elaborate an ICD revision proposal. The revised chapters currently circulating among experts for review are Haematology, Endocrinology, Nutrition, Metabolism and Immunology, Neurology and Malformation. Revised chapters follow a primarily clinical approach, only secondarily an aetiological one up to the gene level. When several names are possible for a disease, descriptive names formed in accordance with a clinical approach are preferred. Every entity is assigned a unique identifying number. Rare diseases affecting several body systems are included in every relevant chapter, as ICD11 will be polyaxial: a main code is also selected for linearisation purposes, according to the severest involvement and/or the specialist most likely to manage the disease. In some cases, the choice is open for debate. Input from the Rare Disease Community is expected.

P01.51 An overview of rare diseases research in Europe based on data from the Orphanet database.**N. Martin, N. Doulet, V. Hivert, S. Aymé;***Orphanet - INSERM SC11, Paris, France.*

Orphanet compiles 6500 research projects. This information has been analysed to identify areas needing collaborative research projects and to target future calls for proposals. The analysis of the distribution of number of diseases by number of treatments in development shows that most RD have no more than 3 products in development, whereas 53 RD have over three such products. Similar analysis results were obtained for clinical trials, marketed drugs, patient registries and pre-clinical/epidemiological/basic research. Some of the diseases over-represented upstream in the process of R&D (with a treatment on the market or drugs in development) are well represented regarding ongoing research (Cystic fibrosis, pulmonary arterial hypertension, some rare cancers). Diseases with a higher prevalence are anticipated to have more treatments in development: this assumption is not backed up by our data analysis. The most developed areas where the ones for which large European networks and consortium have been established as it is the only way to achieve the critical mass necessary in terms of resources and expertise. However the sustainability of the structures which have been created during the development of these collaborations, such as patient registries, biobanks, and technological platforms, is very difficult to ensure to date. The EC could be involved in the financing of the coordination and maintenance of these structures through a specific call for proposals. This work was supported by the European Union's Seventh Framework Programme (HEALTH-F2-2008-201230).

P01.52 Review of Retinoblastoma families and RB1 mutational analysis in the West of Scotland genetics service**S. Gibson, V. Murday;***Ferguson Smith Centre for Clinical Genetics, Glasgow, United Kingdom.*

Rare inherited diseases can cause challenges for genetic services, both clinical and molecular. Retinoblastoma is a rare childhood cancer of the eye. Bilateral retinoblastoma is caused by de novo or inherited germline RB1 mutations. Most unilateral cases are due to somatic mutations. As the germline and somatic forms cannot be distinguished clinically, molecular diagnosis is essential for affected families. It is important that testing is conducted by an experienced laboratory; that results are given to families in a timely manner and at-risk relatives are identified in order to target screening. Identification of a genetic muta-

tion allows for best management of at-risk individuals and prevents unnecessary examination under anaesthetic (EUA), which can be traumatic for the child and family. This review identified families who had attended West of Scotland genetics over the past twenty years with unilateral or bilateral retinoblastoma. We identified 12 families who had stored DNA samples but not had complete analysis. The samples were sent for analysis of RB1 by bi-directional sequencing and MLPA. Germline mutations were identified in six bilateral cases, including one mosaic, and testing is now available to relatives. One bilateral case requires additional analysis by RT-PCR. No mutation was identified in the five individuals with unilateral retinoblastoma and no other known family history. Unnecessary EUA may now be avoided in their relatives and their families reassured. These families highlight the challenges of rare conditions, where results may take many years and families may need support to adapt to new information many years after diagnosis.

P01.53 Conceptual frameworks determine experts' views on clinical use of schizophrenia genetics results**T. Vrijenhoek¹, C. van El², H. G. Brunner¹, A. Geurts van Kessel¹, M. C.****Cornel², J. A. Veltman¹, A. Nelis³;**¹*Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen Centre for Molecular Life Sciences, Nijmegen, Netherlands,*²*Department of Clinical Genetics and EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, Netherlands, ³Centre for Society and Genomics (CSG), Institute for Science, Innovation and Society, Radboud University Nijmegen, Nijmegen, Netherlands.*

Recent developments in microarray technology have enabled large-scale genetic studies on schizophrenia, and have led to the discovery of many risk alleles. This development has been accompanied by promising expectations regarding clinical applications. However, most of the known genetic variants meet validity nor utility requirements for application in clinical practice. Ongoing discussions are not only about *when* or *under what conditions*, but also *if* results from large-scale schizophrenia genetics studies are relevant for clinical use. Based on a literature review and in-depth interviews with experts, we explored the arguments used in these discussions. We have identified four different lines of arguing, which state that high-throughput schizophrenia studies will: (1) eventually reveal clinically relevant data; (2) not reveal clinically relevant data directly; (3) only reveal clinically relevant data when combined with non-genetic (environmental) factors; or (4) eventually be translated into a clinical setting, irrespective of clinical relevance. We show that the experts' perception of the relevance of large scale genetic data predominantly depends on their conceptual framework of the relationship between genes and schizophrenia. This is exemplified by discussions around the development of *direct-to-consumer* (DTC) genetic tests. Furthermore, the experts' conceptual framework determines their perception of 'schizophrenia' itself, which is partly reflected in recent discussions on DSM-V. The challenges resulting from large scale genetic research and the demand from consumers and patients for genetic testing call for a solid infrastructural and translational strategy for schizophrenia genetic research.

P01.54 Screening Tests for Genetic Counselling in TURKEY**Y. Erdem¹, F. Teksen²;**¹*Ankara University Faculty of Health Sciences, Ankara, Turkey, ²Ankara University Faculty of Medicine, Ankara, Turkey.*

Turkey is a country where consanguineous marriages are quite common. Approximately one-fourth of marriages in Turkey is consanguineous and as a result, the incidence of autosomal recessive diseases are very high.

In order to avoid the recurrence of these diseases, Ministry of Health has started screening programmes. For instance, in 1995, a premarital screening programme aiming to reduce the incidence of thalassemia major at 33 city center; in 2002, "Phenylketonuria (PKU) Screening Programme"; in 2003, national newborn hearing screening program (NNHSP); in 2006, congenital hypothyroidism screening program; in 2008, a metabolic disorder, Biotinidase screening programmes were established.

Beta-thalassemia prevalence in the country is 2.1%. There are approximately 1.300.000 carriers and 4000 patients in Turkey. The frequency of PKU is 1 in 4500 live births and 1 in 10,000 live births in the United States comparatively. The newborn hearing, PKU, hypothyroidism, and Biotinidase screening programmes were applied nearly to 95.1% of all

newborns in last five years according to Ministry of Health report. In conclusion, it was observed that, premarital screening is a very useful tool for detecting carrier couples and an effective way of controlling the recurrence of some of the autosomal recessive genetic diseases. Also, there is the necessity to newborn screening programmes. In addition to screening programmes, the establishment of Genetic Counseling Services is also very important and the employment of high quality educated genetic counselors in these centers is of great value as they have critical roles in the prevention of inherited diseases.

P01.55 Prevalent mutant alleles in connexin 26 (GJB2) to romanian children with autosomal recessive nonsyndromic deafness

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Inherited hearing impairment affects about 1 in 1000 newborns. Up to 50 percent of all patients with autosomal recessive nonsyndromic deafness in different populations have mutations in the gene encoding the gap-junction protein connexin 26 (GJB2) at locus DFNB1 on chromosome 13q12.

We performed mutation screening of 50 probands for GJB2 in non-syndromic hearing loss families, including those with cases of sporadic deafness, which were compatible with recessive inheritance.

35delG and 167delT are two GJB2 alleles that cause nonsyndromic recessive deafness, and carrier rates for these mutant alleles may be as high as 4% in some ethnic populations (Estivill et al. 1998; Morell et al. 1998).

However, a large fraction (20 to 63 percent) of patients with GJB2 mutations have only one mutant allele; the accompanying mutation has not been identified.

In our study, these results highlight the usefulness of Cx26 mutation screening for genetic counseling and suggest importance of entire sequencing of the gene responsible for DFNB1.

P01.56 A practice framework for promoting appropriate reporting and use of molecular genetic test results: combining education, test result reporting, and information resources

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Clinical molecular genetic testing for heritable conditions is a growing segment of laboratory medicine. However, studies suggest that clinicians are challenged in staying up to date with knowledge about the genetic tests used in their medical practices and this increases the risk that patient care will be compromised. To address this concern, we propose a practice model to promote appropriate clinical decision making that combines education, effective laboratory reporting of test results, and access to credible and useful information relevant to the test ordered and result reported. This model is based upon several studies that are now complete and will be summarized. Some of the issues that the model addresses include 1) variations in the use of terminology and nomenclature, 2) understanding the relevance of the indication(s) for testing, 3) effectively communicating the limitations of the test method and result interpretation, 4) integrating knowledge of family history, race ethnicity, and other factors that are necessary for interpreting the test result, and 5) using the test result report as a tool for clinical decision making. An interactive, multimedia education program targeting clinicians has been developed to support this practice model. We facilitated collaboration between clinicians and laboratory professionals who evaluated test result reporting processes and developed a clinician-friendly laboratory test result report that has been adapted to a number of clinical scenarios. Combining these tools with access to readily available information resources should prove useful to practicing clinicians by enhancing professional competency and promoting good clinical decision making.

P01.57 Providing access to genetic variation: The SNP & SEQ Technology Platform at Uppsala University

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The SNP & SEQ Technology Platform (www.genotyping.se) performs SNP genotyping and next generation DNA sequencing (NGS) as a service to academic groups in Sweden and the other Nordic countries and as partner in international collaborative projects. The platform offers genotyping services to research projects from single SNP-markers to genome-wide panels of 1 million SNPs in hundreds or thousands of samples, both for association studies and analysis of copy number alterations and DNA-methylation. The genotyping services include all steps from target selection and assay design, to production scale genotyping and quality assessment of the data. Small panels of 1-12 SNPs are analyzed by fluorescent single base primer extension, medium multiplex genotyping is performed by the Golden Gate assay, and highly multiplexed and genome-wide genotyping by the Infinium II assay (Illumina). Sequencing services are provided for all NGS-applications using two Genome Analyzer_{IIx} instruments (Illumina). We have developed a lab-data system for handling and storing information on samples, markers and genotypes, and for quality control and delivery of the genotype data. The genotyping process is accredited according to the European ISO/IEC 17025:2005 quality standard. For storing and analyzing NGS data using computer cluster hardware we collaborate with UPPMAX (Uppsala Multidisciplinary Center for Advanced Computational Science). Most of the completed genotyping and sequencing projects have studied human complex diseases but several projects in other species in evolutionary studies and comparative genomics have also been conducted. For a complete list of publications to which the SNP & SEQ Technology Platform has contributed, see www.genotyping.se.

P01.58 Ethical Aspects of Prenatal Testing for Neurodegenerative and Nervous-muscle Hereditary Diseases in Sakha (Yakutia)

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Genetic counseling of total 38 pregnant women at risk for spinocerebellar ataxia type 1 (SCAI, n=26) and muscular dystrophy (MD, n=12) have been carried out. All individuals were sakha nationality; women at risk for SCAI significantly more often had higher education than women at risk for MD (76.9% and 33.3%, p<0.05). Also, pregnant women at risk for SCAI were more likely than individuals with MD to perceive that prenatal diagnostics would influence their life. In SCAI group 14(53.8%) women requested prenatal testing then in MD group -10 (83.3%). Pregnant women at risk for SCAI more often didn't want prenatal testing than in MD group (46.2% and 16.2% respectively, p<0.05). The relation of women at risk to prenatal diagnostics can be caused a number of the reasons. The first reason is poor information about prenatal diagnostics, confirmed by questionnaire's data of the sakha rural population. The second one is financial reason, so as not every rural family has a possibility to get to Yakutsk for a genetic counseling and DNA testing. Another possible reason is the ethical features of sakha character: in interpretation of such a philosophical category as a destiny, that is patience in confronting of destiny's strokes such as hereditary disease transmitting in the generation.

P01.59 Teaching genetic counseling to first year medical students

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Should genetic counseling be taught or is it encompassed in the overall ethical concepts first year medical students bear among their knowledge? Philosophers have assumed that morality is an inborn human trait and science has nowadays established the cortical regions which are activated while making explicit right or wrong judgments as well as while facing situations containing implicit moral issues. Whether a result of specific inborn brain development or interneuronal connections, a question raises: „Can genetic counseling issues prove that

their ethical aspects show a tendency of being either inherited or already gained through learning until becoming a student?" After having read an article about the discovery of the CF gene, students had to answer 3 questions motivating their opinion. They were asked if considering right or not, that persons with genetic disorders took part in the researches concerning their disease, acknowledging that genetic disorders are rare and often severe; then, if in case of monogenic diseases with known gene anomalies they believed (thought) a screening in the teaching units to detect carriers of autosomal or X-linked genes could now cause in Romania unhappiness, discomfort, panic, because those persons would feel 'labeled' as 'ill' in front of friends and society, in general; lastly, if they considered useful or not the premarital carrier state detection. No discussions had taken place about genetic counseling during the workshops (and lectures), the results reflecting only the students' personal views.

The pitfalls of the study and the students' answers are discussed for asserting the conclusion.

P01.60 Co-presence of deletional mutations on alpha globin genes in the beta thalassemia carriers in Tehran

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The exact determination of gene defect for thalassemia carriers is essential for premarital screening. It will also help the genetic counselor to advise the family accordingly. Among 142 blood samples from PND (prenatal diagnosis) center of Pasteur Institute of Iran which had exact mutation on beta globin gene, we performed simultaneously some molecular experiences such as Multiplex gap-PCR, ARMS-PCR and sequencing for detecting probable mutation on their alpha globin gene cluster.

All samples (142) had 25 different mutations on their beta globin gene, but just one mutation on alpha globin gene in 16 samples successively identified. 15 samples were heterozygote and one sample was homozygote for deletional alpha mutation named- $\alpha^{3.7}$. All these 16 samples had 8 different mutations on their beta globin gene, on which 7 samples were IVSII-1 and 3 samples were IVSI-5 and simultaneously all 16 samples had - $\alpha^{3.7}$ mutation on their alpha globin gene cluster.

Through these 16 samples, the descriptive statistics of the mean value of HbA2, were 4.2 ± 1.0 , and for MCV: 67.3 ± 5.8 , and for MCH: 20.9 ± 2.7 .

Type and frequency of mutations in population with high prevalence of hemoglobin disorder is very variable. In the several publications from our geographical region, it is obvious that IVSII-1 is the most prevalent mutation in the beta thalassemia patients and the most prevalent mutation among alpha thalassemia carriers is - $\alpha^{3.7}$. Co-presence of both mutation in globin genes in chromosome 16 and chromosome 11, is distinctly - $\alpha^{3.7}$ with IVSII-1.

P01.61 Feasibility of Preconception Screening for Thalassaemia in Indonesia: Exploring the Opinion of Javanese Mothers

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Background: Thalassaemia has become a major health problem in Indonesia. It has been estimated that about 10% of the population carries the mutated gene. However, there are no formal recommendations for thalassaemia screening. This study aimed to explore awareness of thalassemia, and attitude regarding carrier testing among Javanese mothers.

Methods: A Quantitative questionnaire was applied cross sectional, using constructs of the Theory of Planned Behavior.

Results: 180 mothers were invited: 74 had a child affected with thalassemia ($RR=100\%$). Both, attitude towards receiving information about thalassemia ($M=4.08$), and attitude towards carrier testing ($M=4.09$) were high. Awareness was low: mothers whose children were not affected hardly ever heard of thalassemia ($N=106$; $M=1.58$), whereas mothers with an affected child showed high interest in carrier testing ($N=74$; $M=4.02$). Respondents did not perceive control over carrier testing ($M=3.02$), and feared being discriminated against or stigma-

tized ($M=2.39$) if carrier status would be identified. Awareness and attitude towards carrier testing explained future reproductive choice ($R^2=.21$; $p < .001$).

Conclusion: All responding mothers showed high interest in receiving information on both thalassemia and carrier testing. The less educated and/or the more deprived they were, the keener they were on receiving this information. Awareness of thalassemia was low. Even mothers with affected children seemed unaware of the inheritance pattern of the disease, showing the need for genetic counseling in Indonesia. It is therefore advised not only to raise awareness on thalassemia, but to better educate healthcare professionals as well.

P01.62** Usefulness of Factor V Leiden mutation testing in clinical practice

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We have investigated the clinical usefulness of the Activated protein C resistance (APCR)/factor V Leiden mutation (FVL) test by sending out questionnaires to all Norwegian physicians who ordered these tests from our publicly funded service laboratory during a three months period, and 70% (267/383) responded. Indications for testing, patient follow-up, the use of APCR versus FVL tests, and differences in practice between hospital doctors and GP's were examined. We found that 46% of the APCR/FVL tests were predictive tests, ordered for risk assessment in healthy individuals with no previous history of VTE. Among these, 42% of the tests were taken on the initiative of the patient, and 24% were screening tests before prescription of oral contraceptives. Fifty-four per cent of the tests were classified as diagnostic, among which 42% were ordered due to a previous history of VTE, and 22% due to a history of brain stroke or myocardial infarction. The prevalence of FVL heterozygotes was not significantly different between the predictive and diagnostic test groups, 26% and 20%, respectively. Only the predictive tests influenced patient follow-up. Here, physician's advice to patients depended on the test result. In general, the clinical usefulness of APCR/FVL testing was low. Many tests were performed on unsubstantiated or vague indications. Furthermore, normal test results led to unwarranted refrain from giving advice about antithrombotic measures, to the potential harm of the patient.

P01.63 How do clinical geneticists-in-training spend their working hours?

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Aim: To determine how clinical geneticists in training spend their time and to compare this to doctors in other specialties.

Method: Time-motion study. A clinical geneticist-in-training recorded her activities prompted by an alarm clock at ten minutes intervals during five consecutive workdays. Activities were categorised as direct patient care, indirect patient care, administrative tasks, conference participation, external professional communication of laboratory results and test options, research, break, and other.

Results: 235 observations were recorded. The proportion of time spent on different categories of work is shown in table 1. Approximately half of the time was used on patient care, but less than a third of this time was spent directly interacting with patients.

Discussion: Studies of doctors-in-training are sparse, but one large study from Australia documenting the activities of doctors-in-training in internal medicine reported similar time fractions spent on direct and indirect patient. Other studies of how doctors in various specialties spend their time report with few exceptions that less than half of the time is spent on patient care and that most patient care takes place without the presence of the patient (charting, reviewing test results, etc.).

Conclusion: The workday of doctors-in-training in clinical genetics may not differ as much from other medical specialties as commonly perceived.

Table 1

| Category of activity | Proportion of time spent |
|--------------------------|--------------------------|
| Direct patient care | 14 % |
| Indirect patient care | 32 % |
| Administrative tasks | 11 % |
| Conference participation | 8 % |
| External communication | 17 % |
| Research/project | 4 % |
| Break | 6 % |
| Other | 8 % |

P01.64 Professional training for the new era of genetic medicine

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Genetics and genomics are making a dynamic and expanding contribution to mainstream healthcare, for example: the increasing use of pharmacogenetics and companion diagnostic testing to personalise medicine. In addition, genetic knowledge is increasing at such a pace that the rate of data generation is at risk of overtaking the capacity for its interpretation.

Nowgen, a Centre for Genetics in Healthcare, is at the forefront of training provision in the arena of genetic medicine. In collaboration with our colleagues in the Central Manchester University Hospitals NHS Foundation Trust, The University of Manchester and the National Genetics Reference Laboratory, Manchester, we have developed a portfolio of training courses for health professionals, industry professionals and research academics in response to expressed needs for training in these diverse areas.

Here we demonstrate Nowgen's role in training provision, including the following areas: Bioinformatics for clinicians and scientists working in genetic medicine, to help develop skills interpreting genetic data; Genetics for research ethics committees, to provide a tool-kit to support committee members in their ethical review of genetic research studies; Oncology-based pharmacogenetics, which aims to provide an introduction to this area, including the regulatory issues, effect on clinical trial design, diagnostic testing and data interpretation; and Molecular genetics for cytogeneticists and genetic counsellors, which aims to provide attendees with an understanding of molecular techniques and analytical methods used in molecular genetics laboratories. All courses have been developed in close consultation with senior representatives from the relevant professional groups.

P01.65 Clinical characterization and analysis of temperamental traits and impact on their parents in 42 Italian young girl with triple X syndrome.

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We report clinical and temperament evaluation data in a large Italian cohort of young girls with triple X syndrome whose diagnosis was made prenatally between 1998 and 2006 in three Italian Centres. At initial evaluation reproductive and medical histories were collected. Clinical assessment of the child was performed by a clinical geneticist and included a detailed personal history, physical evaluation and auxological measurements. To analyze how parents coped with specific events in the prenatal and postnatal periods, we conducted an interview which included 35 specific questions designed to elicit retrospective judgements on prenatal communication, present and future worries, needs and expectations. In a subset of probands we also administered the formal Italian Temperament Questionnaire assessment test which investigates adaptation, general environment and socialization. This test also assesses the emotional component of temperament. Clinical

results in the affected children are similar to those previously reported with evidence of increased growth in the pre-puberal age and an average incidence of congenital malformation and health needs. Mean age at first word was 15 months, showing a slight delay in language skills which tended to improve by the time they reached school age. Parental responses to the interview demonstrated residual anxiety but with a satisfactory adaptation to and a positive recall of the prenatal counselling session.

We believe that an integrated approach to prenatal counselling is the best way to manage the anxiety and falsely imagined consequences which parents feel after being told that their fetus bears such a genetic abnormality.

P01.66 Influence of associations of patients in rare diseases (Williams Syndrome and Smith Magenis Syndrome) in Clinical Research. Research Centers of reference

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In rare genetic diseases research it is a critical issue to build national and international networks of study. Platforms providing the necessary logistics to carry out the proposed work of researchers and clinicians are also needed, due to geographic dispersion. This usually occurs because of the low incidence of rare diseases. Logistic platforms integrating information and centralized federations of rare diseases allow associations concerned to coordinate their activities, ranging from research to therapeutic activities, including leisure and family entertainment. Also, they allow to overcome the barrier of geographical dispersion.

In this context, the Association of Williams Syndrome of Spain has a long tradition in the collaboration with researchers. By other hand, the Spanish Association of Smith Magenis has been recently created. Both associations have joined together to promote research, integrating professionals who study these diseases, in order to develop different projects and clinical programs. They found strategic support in the Regional Center for Rare Diseases (CREER) that allows cooperation among associationism and research. The Spanish Federation for Rare Diseases also supported them, and also another specific centers of genetics as the Galician Public Foundation of Genomic Medicine did so.

This allows to establish a real national internal network of detection and early diagnosis of these diseases, facilitating research. It also helps to develop a better understanding of the neurocognitive, clinician and educational profiles affected and to publish specific materials for different diseases.

The main objective of this paper is to present the existing networks in Spain studying rare diseases.

P01.67 Role of genetic family testing in Wilson disease.

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Wilson's disease (WD) represents a severe hereditary disorder that predominantly affects liver and brain which in case of late revealing and inefficient treatment or its absence leads to severe disabilities, demands considerable financial expenses and even might be fatal. Affected individuals may require health, financial and personal care for 10-15 years. This care is frequently undertaken by family members.

We investigated Moldavian families with WD for identification of mutations at members of families for the purpose of carrying out of preliminary presymptomatic therapy.

We analyzed 34 Moldavian WD patients and family members who were genetically confirmed. In 8 (23,5 %) cases we revealed diseases in siblings of probands on presymptomatic stage, that given us a chance to carry out required therapy and to avoid appearance of clinical WD symptoms and physical disability in these patients.

Disease identification in 23, 5 % presymptomatic patients has a major importance for diagnosis of WD in Moldova. These cases highlight

the importance of accessible and accurate information about rare diseases. Genetic counseling in WD family and molecular testing has a major practical importance for diagnosis what due to early revealing of patients without clinical symptoms of disease gives a chance to begin appropriate therapy in time, to avoid physical disability and to keep a life in such patients.

P01.68 Written information to patients in clinical genetics: what's the impact?

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In all countries of the European Union, oral information must be given to the patient. Written information is generally optional, but physicians are tending more and more to send a copy of the clinical report to the patient. In this study, we aimed to evaluate the impact on patients of sending them written information after a clinical consultation in the genetics department. During a three months period, two geneticists and one genetic counselor offered to send each patient a copy of the letter sent to their general practitioners. A questionnaire was sent with this copy. 375 patients were seen, 60% of the questionnaires were sent back. Of these, 99% showed that this practice was considered a good idea, and 82% reported that the letter reflected the clinical aspects well. 72% thought that receiving this letter improved their understanding, and only 9% found the letter confusing. In general, patients found the words understandable (83%) and only 2% were shocked. 58% would have preferred a letter sent specifically to them, and 63% said that they would have asked their general practitioner to show them the letter. Their main motivation for wanting a copy of this letter was to have the information to pass on to other physicians involved in their health in the future, or to have information concerning the family. There were no significant differences depending on the physician, the indication for the clinical consultation, the age or gender of the patients, or their level of general understanding.

J01.1 Early Diagnosis and Screening of Congenital Heart Anomalies

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There are many cases of congenital heart anomalies missed at the time of birth. Literature demonstrates that CH anomalies are the second most common birth defect in many countries. Despite this fact, our previous study showed that the prevalence of CH anomalies is the fifth most common one indicating that many of these defects are not diagnosed at the time of birth. The aim of this study was to estimate the missing frequency of CH anomalies at the time of birth. The population of the study was 185650 births (183579 live births and 2071 still births) in the northwest of Iran covered by Tabriz Registry of Congenital Anomalies (TRoCA). A total of 451 children with CH anomalies were studied in this region from 2000 to 2009. The expected prevalence of CH anomalies at birth was estimated to be 24.2 per 10000 births (CI 95%: 22.1-26.5) while a prevalence of 9.2 per 10000 births (CI 95%: 7.8-10.6) was observed at the same time/place. This indicated that 59.1 percent of children with CH anomalies were not identified at birth ($P<0.05$). the same proportion increased by 13 percent over the study from 2000 to 2009 ($P>0.1$). The result indicated that a remarkable frequency of CH anomalies was not diagnosed at birth because there was no pediatric cardiologist available at birth in the gynecology and obstetrics wards. This may necessitate the presence of pediatric cardiologist at the time of delivery or soon after birth for early diagnosis and screening of CH anomalies.

J01.2 Marfan syndrome in children

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Pharmacy, Craiova, Romania, ⁵University Hospital, San Carlos, Madrid, Spain. A mutation of the fibrillin-1 gene gives an inherited multisystemic connective-tissue disease, called Marfan syndrome. It is characterized by a wide range of clinical manifestations. The major mortality generators are the cardiovascular manifestations: annuloaortic ectasia, aortic dissection, aortic aneurysm, pulmonary artery dilatation and mitral valve prolapse. Common musculoskeletal manifestations include: scoliosis, pectus excavatum and carinatum, arachnodactyly and acetabular protrusion. Dural ectasia is a characteristic nervous system affection. In some patients there is also pulmonary and ocular involvement. To improve the life expectancy and quality, an early identification and treatment of these clinical aspects is a must. Geneticists play a key role in Marfan syndrome diagnosis. For a better patient care, a comprehensive diagnostic is driven by a prenatal genetic counseling and the knowledge about the various manifestations of Marfan syndrome.

J01.3 Genetic Screening in Europe

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Background: Genetic screening has been defined as any kind of test performed systematically for the early detection or exclusion of a genetic disease, genetic predisposition or resistance to a disease, or to determine whether a person carries a gene variant, that may produce disease in his or her offspring.

Methods: The data were collected from answers of experts via a self-designed questionnaire, which addressed the conditions screened, screening methods, organisational aspects of screening programmes, and conditions screened in prenatal, population-based carrier, and cascade screening programmes, as well as data from websites of national screening authorities and societies and several organisations dealing with neonatal screening issues.

Results and conclusion: The current (2006-2008) status of genetic screening and the organisation of genetic screening programmes in selected European countries is presented in this survey as a background for future attempts of harmonizing standards and procedures of genetic screening, an explicit aim of the European Network of Excellence, EuroGentest (www.eurogentest.org).

P02 Clinical genetics and Dysmorphology

P02.001 Interstitial deletion of 10p12-p11: A novel micro-deletion syndrome associated with mental retardation and a distinct phenotype

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The ability to detect cryptic unbalanced rearrangements in patients with syndromic mental retardation has improved considerably with the clinical implementation of genomic microarrays. Here we report on the molecular karyotyping of three unrelated patients with partially overlapping microdeletions at chromosome 10p11.23-p12.1 ranging from 0.99 Mb to 4.03 Mb in size. The smallest region of overlap (SMO) between the deletions is 400 kb. Yet another patient has previously been described with a 10 Mb deletion, partially overlapping with our three patients {Shahdadpuri, 2008 #1}. All four patients have a developmental delay and growth retardation.

Apart from the SMO, the genomic region could be divided into three separate intervals when related to phenotypic expression and symptoms. Together with the previously reported patient our study suggests that the deletions may represent a novel micro-deletion syndrome with a distinct clinical expression associated with haploinsufficiency for genes in this region.

Reference

Shahdadpuri R, de Vries B, Pfundt R, de Leeuw N and Reardon W. American Journal of Medical Genetics Part A 146A: 233-237 (2008)

P02.002 Deletion of 13q32.3-34: A genotype-phenotype correlation of three cases

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Background: Partial deletions of chromosome 13 are rare. Based on their clinical and cytogenetic features patients have been categorized into three groups: 1) deletions proximal to 13q32, 2) deletions involving 13q32; and 3) deletions of 13q33-34. Of these, group 2 has the most severe phenotype, including, among others, dysmorphology of the brain, eyes, gastrointestinal tract and distal limbs.

Methods: We have collected clinical data and performed molecular cytogenetic mapping of three cases with de novo deletions of 13q32.3-34, using metaphase FISH and genome-wide array CGH.

Results: In case 1, FISH analysis mapped the deletion to range from 99.53 Mb to pter on chromosome 13. The clinical symptoms included, among others, developmental delay, an intracranial dural arteriovenous fistula and low levels of clotting factors 7 and 10. In case 2, FISH analysis mapped the deletion to span from 107.74 Mb to pter. The clinical symptoms included, among others, developmental delay, microcephaly, hypertelorism and low levels of clotting factors 7 and 10. In case 3, array CGH mapped the deletion to range from 101.07 Mb to pter. The clinical symptoms included, among others, developmental delay, microcephaly and hypotonia.

Conclusions: We present three cases with 13q32.3-34 deletions, characterized at the molecular cytogenetic level. In the deleted regions, several candidate genes responsible for the diverse phenotypes of the patients could be identified. Characterization of additional patients harboring similar distal deletions might lead to better, and clinically more useful, description of the 13q deletion syndrome.

P02.003 Narrowing down the minimal critical deletion region of recurrent 16p11.2-p12.2 microdeletion syndrome

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Microdeletions in 16p11.2-p12.2 were recently described in a number of patients exhibiting mental retardation, speech delay, developmental delay, hypotonia, dysmorphic features, and recurrent infections. Yet, due to the size of the deletions (~ 8Mb) and the number of genes contained it was difficult to link the deleted genes to the phenotype.

Here we report the case of an 11-year-old girl with muscular hypotonia, speech delay, ptosis, recurrent infections and dysmorphic features similar to those reported in patients with microdeletions in 16p11.2-p12.2. A muscle biopsy at the age of 1 year showed unspecific changes, whereas the altered structure of the mitochondria was most striking. However, biochemical investigations for mitochondrialopathies were inconclusive. Array-CGH analysis revealed a 205kb microdeletion in 16p11.2, overlapping the deleted regions in the 6 patients recently reported. The microdeletion in our patient was inherited from the mother, who following anamnesis and physical examination, also exhibited mild muscular hypotonia, ptosis, and recurrent ear infections. The microdeletion contains only three disease causing genes: TUFM, ATP2A1 and CD19. We hypothesize that the muscular hypotonia could be due to a deletion of ATP2A1 and the recurrent infections might be associated with CD19 deficiency. This theory is further supported by the observation that only one patient reported so far did not exhibit muscular hypotonia, and this was also the only patient with a microdeletion not including ATP2A1. However, further investigations to confirm the central role of ATP2A1 and CD19 in the phenotypic presentation of patients with 16p11.2-p12.2 microdeletions are necessary.

P02.004 16p13.3 microduplication syndrome : report of 4 cases and further phenotype delineation

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Duplication of the critical Rubinstein Taybi deletion region on the chromosome 16p13.3 have recently been proposed to be a cause of a recognizable syndrome, characterized by normal to moderately retarded mental development, normal growth, mild arthrogryposis, mild abnormalities of the hands, characteristic facial features, and occasional anomalies of the heart. Up to date, only 13 patients with an interstitial duplication of the 16p13.3 region encompassing the CREBBP gene have been reported.

We report 4 new cases with such 16p13.3 microduplication. They present mental retardation (4/4), normal growth (4/4), mild arthrogryposis anomalies (2/4), and no anomalies of the heart. Remarkable facial features such as microretrognathia (4/4), broad nose (4/4), dolicocephaly (2/4) were noted, and also frequent eye involvement (4/4) with upslanting eyes with narrow palpebral fissures (4/4), ptosis (3/4), bilateral epicanthi (3/4). Minor anomalies of the extremities- hands and feet- were always found (4/4), with frequent involvement of the thumb: proximally adducted thumbs (3/4), adducted thumbs (2/4). Several other occasional findings reported before were also noted : cleft palate (2/4) bifid uvula (1/4), inguinal hernia (2/4), hyperconvex nails (2/4) and hammer halluces (1/4)/short halluces (1/4). One patient also presents retinal hypopigmentation. A detailed clinical description of the 4 patients with review of the literature will be made, confirming the recognizable microduplication syndrome, and helping to delineate the associated phenotype.

P02.005 Microduplication of 17p13.3 including YWHAE in a patient with strongly increased nuchal translucency as a fetus, and postnatal abnormal phenotype.

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Background: Deletion of 17p13.3 causes Miller-Dieker syndrome. Duplications in this region have been described with a variable phenotype depending on which genes are included. The genes PAFAH1B1, encoding LIS1, and YWHAE seem to be important for the genotype-phenotype correlation. Duplications including the YWHAE gene but not PAFAH1B1 have been described in a few patients, with common features being facial dysmorphism, macrosomia, and mild developmental delay. Individuals with duplications including PAFAH1B1 have no particularly dysmorphic features, but more severe developmental delay, and brain defects.

Case: Our patient is a boy, 2 years old. The prenatal ultrasound scan showed an increased nuchal translucency of 7.5 mm, no malformations were seen. A normal male karyotype was found. At the age of 2 years the patient showed increased growth and psychomotor retardation. He was hypotonic, had a bad temper, and expressive and impressive language delay. Facial dysmorphism included epicanthus, low set ears, smooth philtrum, thin upper lip, and short nose. Array-CGH showed a de novo 1.02 MB duplication on chromosome 17p13.3. The duplication included the YWHAE gene, but not the PAFAH1B1 gene.

Conclusion: A few cases of duplication 17p13.3 have been described with variable phenotype depending on whether YWHAE or PAFAH1B1 is involved. We here describe a patient with duplication including YWHAE with very similar features to previous cases, but also with an increased nuchal translucency, thus extending the phenotypic spectrum. It can be considered in pregnancies with very high nuchal translucency combined with normal karyotype to investigate for duplications in 17p13.3.

P02.006** The 17q21.31 microdeletion syndrome

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The 17q21.31 microdeletion syndrome is a novel genomic disorder with a prevalence of around 1/16,000. We present an update of the delineation of the phenotype based on the clinical description of 47 individuals with the recurrent 17q21.31 deletion and call for an international collaboration to collect information on this emerging syndrome (www.17q21.com). In our cohort of patients the most consistent features are hypotonia and mild to severe global psychomotor developmental delay. The facial dysmorphisms include a high/broad forehead,

long face, upward slanting palpebral fissures, epicanthic folds, an abnormally shaped nose, large prominent ears and an everted lower lip. Abnormal hair pigmentation and texture is also frequently present. The facial phenotype may evolve with age, leading to coarsening and elongation of the face. Short stature, pectus excavatum, spine anomalies, dislocation of the hip(s), long slender fingers and slender lower limbs, and positional deformities of the hand/feet are also reported. A history of epilepsy is noted in ~50% of all cases. Other serious abnormalities include atrial and ventricular septal defects, kidney/ urologic anomalies, and cryptorchidism. Our data show that the 17q21.31 microdeletion syndrome is a clinically well recognizable genomic disorder. Long-term studies, however, are required to further define the syndrome and to determine the prognosis in adult patients.

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P02.007 Loss of Cytochrome P450 17A1 Protein Expression in a 17 α -Hydroxylase/17,20-Lyase-Deficient 46,XY Female Caused by Two Novel Mutations in the CYP17A1 Gene

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17 α -hydroxylase deficiency (17OHD) is a rare form of congenital adrenal hyperplasia (CAH) caused by mutations in the *CYP17A1* gene. This condition shows considerable clinical and biochemical variation. Molecular characterization of novel mutations in the *CYP17A1* gene and detailed study of their structural, enzymatic, and clinical consequences are required to fully understand enzyme behavior. Here, we present the first molecular characterization of two novel mutations in *CYP17A1* in a 15-year-old female Mexican mestizo 46,XY female with primary amenorrhea and lack of pubertal development, and severe hypertension that manifested only after surgery. A complete clinical and biochemical evaluation was compatible with 17OHD. Structural anomalies in the *CYP17A1* gene were discovered by direct automated sequencing, which revealed a novel compound heterozygous K110X/R362H mutation that leads to a complete lack of enzyme activity. Immunohistochemical analyses performed to determine protein expression and localization showed that cytochrome P450 17A1 was completely absent in the patient's testicular tissue. Studies of novel mutations, such as those described here, provide important information that allows us to better understand the effect of a given mutation on enzyme function and to observe the impact of the mutation on clinical phenotype.

P02.008 Three Interrupted Deletions at 1p36 in a Patient with Lateral Ventricular Enlargement, Epilepsy and Corpus Callosum Abnormality

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Monosomy 1p36 is a frequent terminal microdeletion syndrome characterized by distinct craniofacial features, developmental delay, mental retardation, hypotonia, seizures, visual, auditory, structural brain and cardiovascular malformations.

The 1p36 deletion syndrome is likely to be associated with haploinsufficiency of contiguous genes. In previous studies *KCNAB29* (voltage-gated potassium channel β -subunit gene) and *GABRD* (the human γ -aminobutyric acid A receptor delta-subunit gene) were suggested as candidate genes for epilepsy. Genes within the 2.2 Mb smallest region of overlap have also been suggested as potential candidate genes for periventricular nodular heterotopias (PH).

Here we report a case with three consecutive interstitial deletions at 1p36.22-p36.23, 1p36.31 and 1p36.32array CGH. All the three deletions were within the common deletion region (1pter-p36.23) of classical monosomy 1p36 patients. The present case has mental retardation, developmental delay, dysgenesis of the corpus callosum, epilepsy, lateral ventricular enlargement and facial dysmorphism. In this study we compared the phenotypes and the deletion sizes of the present case with previously published cases. Our study suggests a new critical re-

gion at 1p36.31 for dysgenesis of the corpus callosum and epilepsy.

P02.009 Severe Lysosomal Lipid Storage Disease of the Liver in Monosomy1p36: New Presentation Extending the Phenotype

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Background /Aim : Monosomy 1p36 has been increasingly recognized as a distinct chromosome deletion syndrome in the past few years. Monosomy 1p36 is mostly associated with severe mental retardation, developmental delay, behavioral difficulties and self-injury. There are several distinct dysmorphic features, including large anterior fontanel, microcephaly, brachycephaly, deep-set eyes, flat nose, nasal bridge and pointed chin. In contrast to the "classical" phenotype, several children with a 1p deletion have had overgrowth and hyperphagia with a clinical presentation similar to Prader -Willi syndrome (PWS).

Methods: Here we describe an 11-year-old girl with 1p36 deletion demonstrating the classical dysmorphic features, having developed an uncontrolled voracious appetite and severe truncal obesity. Gastroenterological evaluation revealed elevated liver enzymes. Liver biopsy disclosed severe fatty liver: in addition to medium-size triglyceride droplets, hepatocytes showed excessive lipofuscin accumulation. A most unusual feature was the presence of frequent, extremely large lipolysosomes, never previously reported in this condition.

Results: Oligonucleotide-based microarray analysis was performed using a 105K-feature whole-genome microarray. It showed copy-number loss of 177 oligonucleotide probes from the short arm of chromosome 1 at 1p36.33p36.32, approximately 2.2 Mb in size.

Conclusions: We suggest that the chromosome segment 1p36.33-36.32 harbour a critical region for the manifestation of obesity and hyperphagia. We also suggest that monosomy 1p36 syndrome should be considered in patients with hypotonia, developmental delay, obesity, hyperphagia, behavioral disorders, learning difficulties and a negative genetic test for PWS, even in the absence of the striking facial features of the syndrome.

P02.010 BAC array analysis detects microdeletions on chromosome region 20p12.1 in two unrelated individuals with MR/ MCA syndrome

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One patient with a *de novo* deletion spanning a 250kb region of 20p12.1 and clinical features of Kabuki syndrome was described previously (J Med Genet 2007 44:562-569).

Here we report a detailed clinical and molecular investigation of two unrelated individuals, a 15-years-old and a 7½-years-old boys with respectively paternally and maternally inherited overlapping deletions of 20p12.1, detected by BAC array CGH at 1 Mb resolution. Clinically they both presented with characteristic dysmorphic features, dental abnormalities, gum hypertrophy, brain malformations and severe mental retardation. Common dysmorphism include sloping forehead, arched eyebrows, long palpebral fissures, long eyelashes with unusual growth pattern at the lower eyelid, short philtrum, open-mouth appearance, high palate, posterior rotated ears with massive earlobes. Failure to thrive during infancy, periorbital and generalized oedema, hallux valgus were noted. Additionally, the 15-years-old patient presented with nasolacrimal duct stenosis, single palmar crease, thenar hypoplasia, cubitus valgus, pes planus, stereotypic movements. The 7½-years-old boy developed a short stature (SD-4.7) and microcephaly (SD-2.5). He showed epilepsy, retinal pigment changes, cataract, myopia, bilateral congenital hip luxation and cryptorchid testes. A deletion of the BAC clone RP5-855L24 (14609853-14755918 bp, HG18) was detected in all cells with the 15-years-old patient and his father and as well as with the other and his mother.

The similar clinical presentation in both individuals suggests the causative effect of the aberration. Epigenetic factors could be potential reasons for the healthy parents carrier. To our knowledge these are the first two cases reported with microdeletions 20p12.1 and inherited from the healthy parent.

P02.011 *Drosophila* as a model organism to establish a genotype-phenotype correlation in 2q23.1 deletion syndrome**

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In general, patients with a 2q23.1 microdeletion are mentally retarded and present with speech delay, short stature, microcephaly, seizures, coarse facies, sleep disturbances and behavioral problems. These features may lead to the initial clinical impression of Angelman, Rett and/or Smith-Magenis syndromes.

The overlapping 2q23.1 deletion region in all reported patients comprises one gene, *MBD5*, which is a member of the methyl CpG-binding domain protein family. Another gene in this region, *EPC2*, is also deleted in the majority of patients, who appear to have a more severe phenotype than those with a deletion of *MBD5* only. *EPC2* is a member of the polycomb protein family, involved in heterochromatin formation. Several genes with a role in modulation of chromatin structures are associated with mental retardation. Therefore, *EPC2* might be considered as a mental retardation gene.

In the absence of patients with mutations or deletions of *EPC2* only, we used *Drosophila melanogaster* as model organism to elucidate possible pathogenicity of this gene. Ubiquitous knockdown of the fly ortholog epc induces lethality, mainly during pupal stages. Drosophila also offers the possibility of tissue and cell-specific gene ablation. Epc knockdown in wings and eyes, severely disturbs morphogenesis of these organs. These results point to an important function of epc during development and suggest a significant contribution to the pathophysiology of the 2q23.1 microdeletion syndrome. We will now focus on the role of epc in neuronal function and behaviour.

This study aims to establish the relevance of fly models in understanding gene-phenotype correlations in microdeletion syndromes.

P02.012 Situs inversus totalis with 47,XXX; A case report

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We report a case of a 1,5 year old female situs inversus totalis with 47,XXX. We think that this is the first case in the literature situs inversus totalis with 47,XXX. She is the first child of the family and has no sister or brother. She was taken to our genetic polyclinic because of her puffy face. She had situs inversus totalis and large VSD on her history. We want to see the karyotype of the patient because she had some abnormalities with her phenotype and we know some cases situs inversus with chromosomal abnormalities. Chromosomal analysis performed on a peripheral blood lymphocyte culture showed a 47,XXX female karyotype. Further studies are needed to explain situs inversus totalis connexion with X chromosome abnormalities.

P02.013 Expanding the clinical and neuroradiological phenotype of 6q terminal deletion syndrome: olfactory bulb aplasia and aqueductal stenosis.

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Subtelomeric deletions of chromosome 6q have emerged as a characteristic microdeletion syndrome, characterized by mental retardation, seizures, mild dysmorphic features, and brain anomalies. The latter most commonly include enlarged ventricles or hydrocephalus and aplasia/hypoplasia of the corpus callosum. Thus far, aplasia of the olfactory bulbs and tracts has only been reported as autopsy findings in two patients with a ring chromosome 6 and in one patient with a 6q23-deletion.

We report on two patients with a *de novo* subtelomeric rearrangement involving the long arm of chromosome 6 (6q27) detected by molecular karyotyping using Array-CGH analysis. Both patients had aplasia of the olfactory bulbs and hydrocephalus due to aqueduct stenosis as well as additional brain malformations. The first patient presented with global developmental impairment, facial dysmorphism, and had an unbalanced reciprocal translocation of chromosome 3 and 6, resulting in a 6q27 deletion and an additional 3q29 duplication. The second patient presented with complete anosmia as the only clinical symptom and showed no signs of delayed physical or mental development.

Thus we conclude that there is considerable variability in the phenotypic spectrum of the disorder and that the degree of developmental impairment does not correlate with the extent of brain findings. Therefore the "6q Terminal Deletion Syndrome" might actually be underdiagnosed as it can manifest by anosmia only. Moreover, since olfactory bulb aplasia seems to belong to the phenotypic spectrum of the 6q terminal deletion syndrome we suggest screening these patients for this malformation by high resolution brain imaging.

P02.014 A case report of Achondroplasia (AD inheritance) in a family with consanguineous marriage(both parents are not affected).

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An eight years old boy with clinical manifestations including: megalcephaly, short stature (height = 108 cm, weight = 24), low nasal bridge, prominent forehead, lumbar lordosis, short tubular bones and radiologic manifestations including: rhizomelic dwarfism, V shape deformity, narrow sacrosciatic notches, small iliac wings, short trident hand. He is the result of a consanguineous marriage (second cousins). In the molecular study, he has G1138A mutation in FGFR3 gene in heterozygote form.

His unaffected parents (33 years old woman and 39 years old man) came for preconceptional genetic counseling because of their affected child and consanguineous marriage and they are candidates for prenatal diagnosis because of the probability of gonadal mosaicism.

P02.015 Unbalanced translocation t(1;5)(p31.3;q23.2) in a child with congenital malformations and a disorder of glycosylation (CDG) affecting ALG6 gene, and characterized by SNP and CGH arrays

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The genetic study of a 3 year old child with mild psychomotor delay, joint hyperlaxity, talipes valgus, flat frontal hemangioma, total agenesis of the corpus callosum and some dysmorphic features, revealed a 'de novo' translocation detected by a high resolution karyotype and involving chromosome bands 1p31.3 and 5q23.2. For a better characterization of the breakpoint regions, CGH array (244K) and SNP array (500K) analyses were performed, both showing a hemizygous deletion next to ALG6, a gene containing 15 exons and involved in defective glycosylation. The CGH array deletion was 3.5 Mb in size and started at exon 10 of ALG6, while the SNP array deletion was centromeric, and ALG6 was not included in the deleted region. Other genes such

PGM1 and LEPR where also lost in both arrays analyses. For a better delimitation of the deletion boundaries, PCR amplification of all ALG6 exons was applied to genomic DNA. No product was obtained for exons 11 to 14 when compared to controls, but the amplification of the terminal 3' exon 15 showed a positive result, suggesting an intragenic homozygotic deletion, undetectable by both arrays. An aberrant transcript was obtained using RT-PCR for the detection of ALG6 mRNA. These results suggest a deletion of ALG6 in this patient, affecting both alleles, in a different and non-Previously described mode of protein inactivation. Products obtained from the detected rearrangement must be further characterized.

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P02.016 Isolated autosomal recessive hypotrichosis in Pakistan caused by P2RY5 and LIPH gene mutations

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Autosomal recessive hypotrichosis (LAH) is characterised by diffuse hair loss without associated abnormalities. Typically, hair thinning starts in early childhood and progresses through adolescence. The expression is variable with differences in age of onset and severity of baldness, as well as in hair density and texture. The clinical variation is observed between, as well as within, families segregating hypotrichosis. Recently, the genetic basis of LAH was shown to involve mutations in the P2RY5 gene on chromosome 13q14.3 (OMIM 609239) or the LIPH gene on chromosome 3q27.3 (OMIM 607365). In the present study, we identified eight unrelated consanguineous families from different areas of Pakistan segregating autosomal recessive hypotrichosis. The families were investigated by homozygosity mapping for linkage to P2RY5 and LIPH. Six families showed linkage to the P2RY5 gene and sequencing revealed a single known missense mutation in five families and a novel insertion mutation in one family. The previously known missense mutation was accompanied by a shared haplotype amongst all affected. The remaining two families showed linkage to the LIPH gene and segregated known mutations. The P2RY5 gene encodes a 344 amino acids long G protein-coupled receptor (GPCR). LIPH produces the ligand for P2RY5, acyl-lysophosphatidic acid (LPA). Both genes are highly expressed in the inner root sheaths of the hair follicle and play an essential role in the maintenance of hair growth and texture. From our results, we suggest that a few ancestral mutations cause isolated autosomal recessive hypotrichosis in the Pakistani population.

P02.017 Carrier frequency for alpha triplication in individuals with normal hematologic indices

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The $\alpha^{3.7}$ deletion is the most frequent α -globin mutation in Iran, one would then expect that the anti 3.7 triplication is common in Iran as well. the frequency of β -thalassemia gene is close to %10 in highly prevalent regions of Iran, so we expect to see frequent co-inheritance of alpha triplication with β -thalassemia. Blood samples were selected from individuals with normal hematological indices (MCV>80, MCH>27) (n=153) in Iran population. DNA extraction from peripheral blood leukocytes was performed by salting out method and multiplex PCR was used to detect alpha triplication. Carrier frequency in individuals with normal hematological indices (n=153) was %3.26. This carrier frequency of α -triplication is relatively high (3-26) in this study. the co-inheritance of this determinant could interfere with genotype-phenotype correlation in minor β -thalassemia carriers, and could cause thalassemia intermedia. further characterization by southern blot or MLPA is needed to determine the allele frequency in our population.

P02.018 Alström syndrome in two sisters.

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Alström syndrome is a rare autosomal recessive multisystemic disorder caused by mutations in the ALMS1 gene. The gene is located on 2p13, contains 23 exons and encodes a protein of 4169 amino acids (Collins et al and Hearn et al 2002). Alström syndrome is characterized by cone-rod retinal dystrophy causing blindness, sensorineural hearing loss and major organ involvement such as dilated cardiomyopathy, hepatic and renal failure. Childhood obesity, type 2 diabetes, hypertriglyceridemia and hypothyroidism are other common features. Clinical manifestations are highly variable even within family members. We report two siblings with a clinical diagnosis of Alström syndrome who presented with early onset rod monochromatism, cardiomyopathy, congenital nystagmus, truncal obesity and mild developmental delay. These 2 sisters are both heterozygotes for a novel pathologic mutation (c.5098A>T) in exon 8 of ALMS1 gene, which causes truncation of the ALMS1 protein. Previous studies suggest that the classical syndrome occurs only in patients with homozygous or compound heterozygous mutations of the ALMS1 gene thus the heterozygosity for this novel mutation does not explain the severity of this disease in our patients. Array-CGH studies for both siblings were normal and MLPA studies of the ALMS1 region are currently pending.

Little is known about phenotype-genotype correlation in Alström syndrome, although patients with clinical symptoms of Alström syndrome were found to have disease-causing mutations in exon 16 (Marshall et al 2007). We hypothesize that rearrangement of the ALMS1 gene in addition to this pathologic mutation is the basis for the severe phenotype of our patients.

P02.019 Ambiguous external genitalia in newborns

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A newborn infant with ambiguous genitalia is a medical emergency, and the choice of gender must take into account the chromosomal and the gonadal sex, the hormonal influence during fetal life, surgical aspects, and the anatomy of the internal genitalia. A newborn with ambiguous genitalia needs prompt evaluation that will permit gender assignment and detection of life-threatening conditions (salt-losing crisis due to congenital adrenal hyperplasia -CAH). The classification of this disorder in newborns is difficult because similar phenotypes could have several different etiologies. In most cases it is impossible to correlate the etiology of the disorder and the appearance of the external genitalia. Diagnostic criteria applied are similar for all (physical exam, karyotype, investigation of hormones and their derivatives, genital ultrasound, radiological examination), but medical and surgical treatment is applied to each patient individually.

We present 2 cases of ambiguous genitalia admitted in our department in the last year. **RESULTS:** In one patients (normal karyotype: 46, XX) the underlying cause of ambiguous genitalia was CAH, while the aetiology of sexual ambiguity in the other case was the chromosomal structural anomalies 46,XY,t(X;21)(p11;q21). **CONCLUSION:** The etiology of ambiguous genitalia is variable. The physician managing these families could minimize the trauma of having a child with unidentified sex by providing appropriate genetic counseling so that the parents can make an early decision. A proper diagnosis requires a team working: pediatrician, endocrinologist, genetician and pediatric urologist/surgeon. Prenatal diagnosis in at-risk families should be considered and appropriate therapy offered to minimize or prevent genital ambiguity.

P02.020 Recurrence of complete arhinia in two siblings with clinical picture of Treacher-Collins Syndrome negative for TCOF1 mutations

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Congenital arhinia is an extremely rare malformation derived from an anomalous embryological development of the nose which normally develops between the third and the tenth week of intra-uterine life. The pathogenesis of this anomaly is not clearly understood. Congenital absence of the nose is often associated with other defects, including ear, palatal and ocular malformations, central nervous system and skeletal anomalies. We report the case of two siblings, a 9-year-old girl and a 2-year-old boy, with congenital total arhinia. Both had a complete absence of external nose and nasal cavities associated with dysmorphic facial features including downslanting of palpebral fissures, maxillary hypoplasia and external ear anomalies. Since congenital absence of the nose has been described in a few patients affected by Treacher-Collins Syndrome, we conducted a molecular study of TCOF1 gene on the mother and first child DNA, which were negative for both point mutations and large deletions (PCR and DNA analysis through sequencing of the exons 1-26 and the contiguous intronic sequences). As congenital absence of the nose is an extraordinary occurrence in a family and most cases are sporadic, the recurrence of arhinia in the same family, as described here, suggests a common genetic and hereditary origin. The consanguinity of parents supports the hypothesis of an autosomal recessive disorder; nevertheless, we can not exclude a gonadic mosaicism or an autosomal dominant inheritance, as we are not able to verify if the father had pathological features.

P02.021 Complex genotypes in arrhythmogenic right ventricular cardiomyopathy patients from North West Spain

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Purpose: Desmosomal genes mutations are detected in nearly 50% of arrhythmogenic right ventricular cardiomyopathy (ARVC) patients from different series. Our objective was to analyze the prevalence of mutations in these genes in an ARVC cohort from North West Spain.

Methods: Twenty no related index cases with ARVC diagnosis were clinical evaluated in a dedicated cardiomyopathies clinic. Sequence analysis of main desmosomal genes (PKP2, DSP, DSG2 and DSC2) was performed from fresh blood samples, taken after written consent. Results: We found 10 different mutations in 13 index patients (65%). Four patients (20%) had complex genotypes: 1 was homozygous for a DSC2 gene mutation, 1 was compound heterozygous (two mutations in PKP2 gene) and 2 were double heterozygous (two mutations in different genes). All mutations were novel and two (R375X, S329RfsX351) were present in more than one patient. Both mutations are predicted to produce a truncated peptide. R375X in DSC2 gene was detected in two patients: one was homozygous and developed early and severe phenotype and the other patient had a second mutation in PKP2 gene (S329RfsX351). Moreover, this PKP2 mutation was found in 6 different cases.

Conclusions: Nearly, 20% of patients could have complex genotypes in the main desmosomal genes. The complex genotypes could develop early and severe phenotype. North West Spain have a highly prevalence (65%) of ARVC patients with desmosomal gene mutations.

P02.022 Further delineation of the cerebral morphology in ASPM-related primitive microcephaly : microcephalia vera is not a small, normally organized cortex !

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Primary microcephalies (Microcephaly Primary Hereditary, MCPH) have often been presented as a developmental disorders resulting in homogeneous reduction of grey matter in the cortex, without obvious developmental defect except neuronal aplasia, occurring in

patients with mild MR, hyperactivity and, occasionally, late onset seizures. ASPM is the most commonly involved gene, explaining roughly 25 to 50% of all cases (MCPH5). Careful clinical, neurological and neuroradiological investigation of 12 patients with homozygous or compound heterozygous mutations in ASPM revealed that 1) phenotype may encompass pyramidal signs and short stature (< -2 SD) ; 2) intelligence may be within normal values (IQ > 70) ; 3) more importantly, simplified gyration pattern with anteroposterior gradient is present in most cases. Migration defects (heterotopias), and infratentorial anomalies are present in some patients. We investigated regional cerebral organization through 3D morphometric analysis of MRI scans in 7/12 patients and showed that 1) reduction of brain volume affects equally white and grey matter. 2) Reduction of cortical volume (and surface) affects more the caudal part of frontal lobes and the rostral part of parietal areas, whereas 3) mediotemporal areas and basal structures are preserved, which is in accordance with preserved mnemonic performances. These data allow us to broaden the phenotype of MCPH and to redefine its diagnostic criteria.

P02.023 Mutations of codon 2085 in the helicase domain of ATRX are recurrent and cause a moderate form of ATRX syndrome

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ATRX syndrome is characterised by the association of profound mental retardation, facial dysmorphism, urogenital abnormalities and alpha-thalassemia. A large number of ATRX mutations have been described as causative in ATRX syndrome, the majority being missense mutations. Most of the 80 missense mutations reported so far, have been described in one or two families with exception of the R246C which was found in 20% of the ATRX syndromes. Here, we described mutations localised on codon 2085, in one of the 2 major functional domains, the helicase domain, and associated with a mild form of the disease.

Among the patients addressed to our lab for ATRX analysis, we found 5 patients from 3 families carrying a mutation on codon 2085, a highly conserved residue of the helicase domain. The substitution was R2085H in 2 families and R2085C in the last one. Clinical data were as follow: the 5 patients have discrete but characteristic facial dysmorphism and present with mental retardation: all of them acquired autonomous walking between 18 and 24 months, three has no speech skill, one can say words, the last one sentences. None of them have genital abnormalities nor seizure. The 3 mothers are carriers and have highly skewed chromosome X inactivation. No mutation of the codon 2085 was found after testing 250 control chromosomes.

In conclusion, missense mutations localised on residue 2085 should clearly be considered as causative mutations even though they lead to moderate and incomplete forms of ATRX syndrome.

P02.024 Detection of genomic imbalances by arrayCGH in two children with syndromic autism.

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Autism is a complex behaviorally-defined disorder of the immature brain. It is not a disease but a syndrome with multiple non genetic and/or genetic causes. Autism spectrum disorders (ASD) are conditions which can be either isolated or syndromic, that is associated with other clinical features such as facial dysmorphism, limb or visceral malformations, and growth abnormalities. Currently, diagnosable medical conditions, cytogenetic abnormalities, environmental factors, and single-gene defects associated with autism, together account for 10-20% of cases.

We report two children with autistic behavior, mental retardation and dysmorphic features. We have used genomic array CytoChip (BlueGnome, Cambridge, UK), covering the entire genome at a median 565 kb, a resolution optimised to detect pathogenic imbalances while mini-

mizing polymorphisms. Array CGH- analysis revealed cryptic amplification of 16p11.2 region spanning 1,362 Mb in first patient and an amplification spanning 1,274Mb of 7p12.3 region in second patient. The microdeletion and a reciprocal microduplication of 16p11.2 region carry substantial susceptibility to autism and appear to account for approximately 1% of cases. 7p12.3 is a region of shared genetic association between ASD and attention deficit hyperactivity disorder (ADHD). These data strongly support the idea that only a whole-genome high-resolution analysis such as array-CGH is able to provide an accurate diagnosis for chromosomal imbalance in patients with ASD. Confirmatory FISH studies with BAC clones are planned for accurate confirmation of CytoChip result.

P02.025** Autism Spectrum Disorders: emerging data from Copy Number Variations analysis

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Autism Spectrum Disorders (ASDs) have a complex and heterogeneous aetiology with a strong evidence of a genetic involvement. To assess the frequency and type of copy number variations (CNVs) in ASD, a cohort of 95 patients has been selected and analyzed by oligo array-CGH with a functional resolution of nearly 100 kb. Array-CGH resulted negative in 57 patients while in 38 at least one rearrangement was identified. A total of 51 rearrangements was identified: 23 deletions and 28 duplications. Among the 38 patients with CNVs the M: F ratio is higher than expected resulting in 6.6:1. Seven CNVs turned to be pathogenic: one *de novo* (del Xq12) and 6 located in known autism susceptibility regions (dup15q13.3, dup16p13.1, delXp22.31, del15q11.2, del11p12, dup17q12). The 7 patients with pathogenic CNVs were all males with intellectual disability (ID). The majority presented with congenital anomalies (MCA) and dysmorphisms (57.1%); none suffered from epilepsy. Among the cohort of 57 patients without CNVs the M:F ratio resulted in 4.7:1, as currently reported in ASDs. ID was present in 96.5%; MCA and dysmorphisms were present respectively in 21% and 63.2%. Epilepsy rate was 19.3%. The detection rate of CNVs in our series of patients is 7.4%. Patients with pathogenic CNVs differed from the cohort without any CNVs for the presence of congenital anomalies, more frequent in the first group. Furthermore, the rate of epilepsy found in the all group was 19.3%, while in the subgroup of patients with pathogenic CNVs (n=7) epilepsy was not detected.

P02.026 Evaluation of clinical findings in 20 patients with Bardet-Biedl Syndrome

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Bardet-Biedl syndrome (BBS) is a genetically heterogeneous disorder characterized by one or more of the following six major features: retinal degeneration, early-onset obesity, cognitive impairment, genitourinary tract malformations, renal dysfunction and polydactyly. According to recent studies, defects in the basal bodies of ciliated cells appear to be the underlying cause of the syndrome. Although BBS was originally thought to be a recessive disorder, it has since been shown that some forms of BBS are passed on via triallelic inheritance.

We retrospectively evaluated 20 patients with BBS because of associated abnormalities. between January 2001 and January 2009. We found both classical and rare findings of BBS in our patients. We identified a patient with persistent urogenital sinus, urethrovaginal fistule together with BBS as rare findings. Our special attention was given to monitoring those patients with renal abnormalities, because renal disease is a major cause of premature mortality in patients with BBS. We found renal abnormalities in 10 of the patients. The renal abnormalities observed ranged from minimal pelvicalyceal dilatation (PKD) to end-stage renal failure. Among of these, four had a polycystic kidney and six had PKD. Due to end-stage renal failure, three of these patients received a renal transplant from a relative. Four of the patients were

still undergoing periton dialysis at the end of the evaluation period. Because of the morbidity and mortality associated with renal abnormalities in BBS, this study demonstrates the importance of long-term follow up so that proper care and treatment can be provided.

P02.027 A micro-duplication of the centromeric domain of the 11p15.5 imprinted gene cluster is associated with loss of DNA methylation and familial BWS

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Beckwith-Wiedemann syndrome (BWS) is an overgrowth disorder caused by a variety of molecular lesions affecting a two-domain cluster of imprinted genes at chromosome 11p15.5. Most cases are sporadic and result from DNA methylation defects at either one of the two imprinting centres (IC1 and IC2). Here, we describe a familial case with a 166 kb duplication of the centromeric domain and loss of IC2 methylation. The proband is an infant with macroglossia, macrosomia, onfocele and hexadactylysm, her mother and uncle presented with abdominal wall defects. The duplication that comprises IC2 and the 5' part of the anti-sense KCNQ1OT1 gene was identified by MS-MLPA (methylation-sensitive multiple-ligation-probe-amplification), confirmed by SNPa analysis and demonstrated to be *in-cis* by FISH. This genomic lesion cosegregates with BWS phenotype and IC2 hypomethylation, but does not affect IC1 methylation. cDNA sequencing of KCNQ1OT1 demonstrated that both the maternal and paternal alleles were expressed in the proband and her mother, indicating loss of KCNQ1OT1 imprinting. The results obtained are consistent with the presence of a maternal IC2 duplication that results in partial hypomethylation at IC2 and activation of the maternal KCNQ1OT1, likely silencing the imprinted genes of the IC2 domain (e.g. CDKN1C). This is the first familial case of BWS with IC2 hypomethylation in which the molecular defect is identified. Maternal 11p15.5 duplications are generally associated with the Silver-Russell syndrome phenotype and include the CDKN1C gene. On the contrary, in the reported case, the duplication does not include any full-length genes, affects IC2 methylation and causes BWS.

P02.028 Microdeletions within the “Imprinting region 2” on 11p15 identify a distinct category of Beckwith-Wiedemann syndrome phenotype at risk for cardiac arrhythmia.

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Beckwith-Wiedemann syndrome (BWS) is an overgrowth condition characterized by clinical variability and genetic heterogeneity. Psychomotor retardation is uncommon and familiar recurrence rare. BWS is caused by disregulation of two imprinting domains (ICR1 and ICR2) on 11p15, ICR2 being involved in most cases. Abnormalities of ICR2 include loss of methylation on the maternal allele, or point mutations of CDKN1C.

We observed a 11p15 microdeletion involving ICR2 in two unrelated females, aged 16 and 19 years, respectively. The deletion spanned about 900 kb in the first case, including CDKN1C, KCNQ1 and additional 15 flanking genes. The size of the deletion in the second patient was 200 kb, encompassing the entire KCNQ1OT1 antisense transcript, ICR2 and part of KCNQ1. Loss-of-function mutations of KCNQ1 can be responsible for long QT syndrome type 1.

Both patients presented with an atypical BWS phenotype, including mental retardation in the first patient, a long QT syndrome in the second and overgrowth and facial dysmorphisms in both. The long QT syndrome was responsible for a cluster of life-threatening ventricular fibrillation episodes, needing an urgent implant of intra-cardiac defibrillator.

Literature deals with only one additional BWS patient with microdeletion of ICR2. However this event might be under-estimated. A search for a ICR2 microdeletion should be performed in each BWS patient with impaired methylation of ICR2, and patients hemizygous for KCNQ1 should be monitored for long QT syndrome. If a ICR2 microdeletion

is diagnosed in female patients, a 50% recurrence risk of BWS is expected in the offspring.

P02.029 Orofaciodigital syndromes: a case series.

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Introduction: Orofaciodigital syndrome (OFD), an inherited syndrome, with varying combinations of defects of the oral cavity, face, and hands, including lobulated or bifid tongue, cleft or pseudocleft palate, tongue tumours, missing or malpositioned teeth, hypoplastic nasal alar cartilage, depressed nasal bridge, bifid nasal tip, brachydactyly, clinodactyly, incomplete syndactyly, and, frequently, mental retardation. They have variable presentations as well as inheritance patterns.

Objectives: To study clinical variability of orofacioldigital syndrome in subjects who were seen in genetic clinic in last 3 years.

Methods: Subjects with OFDs seen in Genetic unit in APC were subjects of this retrospective analysis.

Results: There were 4 cases with OFD with M:F ratio = 1:3 and the consultations were from age of 4 years to 21 years. On database search, the clinical diagnoses made were: OFD I, OFD II and OFD IX in 3 cases each with 4th subject having overlapping features of both OFD II & VI. The last case had lingual nodules, central polydactyly, bifid thumb, cardiac defect and bilateral renal parenchymal disease. Predominant clinical presentations were developmental delay, abnormal facies, digital anomaly and short stature.

Conclusions: Subjects with OFDs can have varying clinical presentation and should be differentiated from craniosynostosis syndromes and oral-cardio-digital syndromes. Digital anomalies may not be present in all. A high clinical suspicion, examination of parents and appropriate investigations are required to arrive at early diagnosis and provide proper genetic counseling. OFD II and OFD VI may represent a clinical continuum of same underlying gene defect.

P02.030 Two siblings with blepharophimosis, psychomotor delay and severe growth failure: a new autosomal recessive blepharophimosis-mental retardation syndrome?

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Blepharophimosis is characterized by a fixed reduction in the vertical distance between the upper and lower eyelids with short palpebral fissures. It is commonly associated to other periocular anomalies that may occur either as isolated features or as part of multiple congenital anomaly (MCA) syndromes. Many syndromic blepharophimosis patients show variable degree of mental retardation and are referred to as BMR (blepharophimosis mental retardation), a term which includes a clinical and etiological heterogeneous group of disorders. We report on two siblings, a 6 year old girl and a 18 month old male, presenting overlapping clinical findings. Striking facial dysmorphisms included upward slanted palpebral fissures, blepharophimosis, telecanthus, hypertelorism, posteriorly rotated ears with over-folded helices and micrognathia. Ectodermal abnormalities included fine hair, sparse eyebrows and thin skin. Both patients had feeding difficulties with gastoesophageal reflux and growth retardation. Psychomotor skills were severely delayed and with no verbal capacity. The male sib displayed low GH levels, while the older sister had low cholesterol and mildly raised TSH levels. Numerous metabolic/genetic investigations, including cholesterol precursors dosage and high resolution array-CGH, were negative. The clinical history of the siblings was uploaded to the Dysmorphology Diagnostic System (DDS) developed by DYSCERNE. Differential diagnosis with BMR syndromes, including Dubowitz syndrome, Marden-Walker syndrome, Ohdo/Ohdo-like syndromes and cholesterol storage disorders was discussed with the DDS experts which concluded that these two siblings likely represent a previously unreported autosomal recessive-BMR syndrome.

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P02.031** Larger cohort of Branchio-oculo-facial syndrome (BOFS) patients: phenotype description and *TFAP2A* genotype findings

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BOFS patients develop distinctive features: abnormal external ear; retro-auricular overlying skin; clefts or pseudocleft; eye involvement (microphthalmia or coloboma) and premature grey hair. Patients may have mental retardation and all type of deafness. *TFAP2A* gene deletion or missense mutations were identified in one familial and 5 sporadic patients (1). Stoetzel et al. confirmed these findings in one family and 3 sporadic patients (2). No precise phenotype criteria was validated to diagnose BOFS. A checklist was elaborated based on the published reports available in BOFS patients. Four familial and 9 sporadic patients with BOFS were included in this study. All patients developed any type of clefts (lip, palate and/or pseudo-). Pre auricular pits/fistula and abnormal external ears were noted in familial presentations as for 6 sporadic patients. Nasolacrimal duct stenosis and malformed nose was diagnosed for two sporadic and one familial presentations. Overlying skin was found in one familial and one sporadic patients. Premature grey hair was noted in three familial and in one sporadic patients. Ophthalmic anomalies were diagnosed in the two familial as in 7 sporadic patients. One family had neurosensorial deafness as 4 sporadic patients. Three sporadic patients developed mental retardation. In all affected patients but one, *TFAP2A* gene sequencing was diagnostic for missense/nonsense mutations or gene deletion. Hotspot mutations in exons 4 and 5 in the gene encoding the transcription factor AP2A were found.

References

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P02.032 A new case of blepharophimosis syndrome (BPES) associated with translocation between chromosomes 3q and 8q

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We report a case of 2 month old girl with short stature, dolichocephaly, blepharophimosis, ptosis and epicanthus inversus. The proband was the first child of a healthy, nonconsanguineous couple. Family anamnesis was negative. Evolution of pregnancy was with hydranios; preterm delivery at 35 months of age. Chromosomal analysis showed an apparently balanced karyotype with translocation between chromosomes 8q and 3q. Genetic testing for parents should be performed in the context of genetic counseling (the karyotype of the parents - in work).

Blepharophimosis syndrome (BPES) is a complex eyelid malformation characterized by four major features: blepharophimosis, ptosis, epicanthus inversus, and telecanthus. The diagnosis of BPES is essentially based on clinical findings.

It is possible that our patient has a contiguous gene defect including at least one locus from chromosome 3q for a type of blepharophimosis, further suggesting that multiple loci exist on chromosome 3q for eyelid development. Cytogenetic rearrangements are rare, estimated to occur in 2% of individuals with BPES. [Beysen et al 2009]

P02.033 Interstitial deletion of chromosome 3q24 to 3q25.33 associated with an unusual brachydactyly, facial telangiectasia and brisk reflexes

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We report a girl with developmental delay, neurological, dermatological and immune features, facial dysmorphism and unusual brachydactyly with a 10.7Mb interstitial deletion within 3q24 to 3q25.33.

The 4th and 5th metacarpals, and 3rd and 4th metatarsals are short, consistent with the overlapping conditions of brachydactyls type D and E. She has marked joint laxity and brisk reflexes, facial telangiectasia and recurrent infections. Growth is normal.

The deleted interval contains 55 annotated genes, but none is an obvious candidate for any of the features observed here.

Deletions of this region of chromosome 3 are rare, with only one further case reported in the literature. No photos of the other case were published, but the description is of 1st and 2nd toes that were unusually long compared with the other toes. This sounds remarkably similar to the findings in our case.

This case is noteworthy because this case represents a new microdeletion syndrome, and we suggest that a key feature of this may be an unusual form of brachydactyly.

P02.034 Brachyphalangy, polydactyly and tibial aplasia/hypoplasia syndrome (OMIM 609945) in a girl born to consanguineous parents

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Brachyphalangy, polydactyly and tibial aplasia/hypoplasia syndrome (OMIM 609945) is a rare syndrome characterized by severe limb malformations, facial dysmorphic features and additional clinical problems. Only seven patients have been reported to date, and the etiology of this disorder is unknown. Autosomal dominant inheritance with variable expression has been suggested based on the presence of minor features in some parents and the fact that neither parental consanguinity nor pairs of affected siblings were reported.

We report on the first patient with this syndrome who was born to consanguineous parents. Neither the mother nor the father, who were first cousins, had clinical features suggestive of a manifestation of brachyphalangy, polydactyly and tibial aplasia/hypoplasia syndrome. The patient had no siblings, and the family history was unremarkable. Clinical problems included brachydactyly of hands and feet, splaying of fingers and toes, preaxial polydactyly of feet, bilateral tibial aplasia, shortened radius and ulna and characteristic facial dysmorphic signs.

Consanguinity in this patient indicates an autosomal recessive etiology, a mode of inheritance that should not be dismissed in genetic counseling as long as the causative gene defect of this disorder remains elusive.

P02.035 A 1.6 Mb deletion of the 19p13.13p13.12 region detected by array-CGH and encompassing the CACNA1A gene is responsible of a non evolutive epileptic encephalopathy with macrocephaly, connective tissue dysplasia and urinary reflux.

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This female patient is the third daughter of unrelated parents. After a pregnancy characterized by a polyhydramnios during the second trimester and an oligoamnios at the end of the third trimester, the delivery occurred spontaneously at 41 WA. At birth, the patient presented a severe hypotonia, marfanoid habitus, hyperelastic skin and severe gastro-oesophageal reflux requiring Nissen surgical treatment. In the first months she presented numerous urinary infections secondary to urinary tract reflux. Dysmorphic features were noticed: high and large forehead, frontal bossing, down slanting palpebral fissures, external strabismus, and arachnodactyly of fingers and toes. The outcome was severe: non evolutive epileptic encephalopathy, absence of speech, absence of walking. She also presented an advance of the stature (+2.5 SD) and the bone age, and a macrocephaly (+2.5 SD). At age 11, a dorsal-lumbar scoliosis was surgically repaired.

All etiological investigations were normal: karyotype with FISH 15q11q13, ophthalmological examination, EEG and EMG, cerebral MRI, muscular and cutaneous biopsy. Array-CGH showed a de novo

19p13.13p13.12 deletion encompassing the CACNA1A gene.

This microdeletion overlapped the deletion recently reported by Auvin (Epilepsia, 50(11):2501-2505, 2009) concerning a patient presenting infantile spasms, neonatal hypotonia, macrocephaly, advance stature and bone age. In the overlapping region of the 2 deletions is located the CACNA1A gene (calcium channel alpha 1A subunit) encoding a sub-unit of voltage-dependant calcium channel. Mutations of this gene are responsible of familial hemiplegic migraine and episodic ataxia type 2. The haploinsufficiency of CACNA1A probably plays an important role in the phenotype of these 2 patients.

P02.036** SHOC2 mutations in patients with cardio-facio-cutaneous syndrome

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Cardio-facio-cutaneous syndrome (CFC), Noonan syndrome (NS) and Costello syndrome (CS) show both considerable phenotypic heterogeneity and overlap, and arise due to mutations in several genes whose products interact within the RAS-MAPK pathway. Whilst *HRAS* mutations cause CS, many different genes (*PTPN11*, *SOS1*, *KRAS*, *NRAS*, *BRAF*, *MEK1* and *MEK2*) cause NS and CFC, and a significant proportion of NS/CFC patients have no mutation identified currently. Patients in whom CS is clinically suspected but no *HRAS* mutation is found are generally considered to have CFC. De novo S2G mutations in *SHOC2* were recently reported in patients with a variant NS phenotype, 'Noonan-like syndrome with loose anagen hair'. This mutation generates a myristylation site, the myristoylated protein then being targeted to the cell membrane, where it interacts with RAS and RAF proteins.

Samples from 84 patients referred for molecular diagnostic testing of *BRAF*, *KRAS*, *MEK1*, *MEK2* and *HRAS* because of a clinical suspicion of CFC or CS, in whom no mutation was found in these genes, were screened for the S2G mutation in *SHOC2*. 11 such mutations were identified, confirming that this mutation is a common and important cause of a CFC/CS/Noonan-like phenotype. Further work to characterise this patient group is underway. These results emphasise the significance of *SHOC2* in germline disorders of the RAS-MAPK pathway and indicate that molecular diagnostic testing for the S2G mutation is likely to yield positive results in groups of patients in whom the diagnoses of CFC, CS or severe Noonan syndrome are being considered.

P02.037 Desmin mutations as a cause of right ventricular cardiac failure affect the intercalated disk.

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Background: Desmin is the main intermediate filament in skeletal and cardiac muscle cells. Abnormal desmin causes skeletal muscle weakness and/or cardiomyopathy. Desmin related myopathy (DRM)(MIM#601419) is associated with dilated (DCM), restrictive or hypertrophic cardiomyopathy. Recently, we observed both DCM and (probable) Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) in three Dutch families carrying an identical desmin (*DES*) mutation. This suggests *DES* mutations to cause cardiomyopathy affecting both the left and right ventricle. Because of this overlap with ARVC, we studied the prevalence of *DES* mutations in (probable) ARVC patients and we studied the cardiac desmosomal composition in *DES* mutation carriers.

Methods: *DES* was screened for mutations in 50 ARVC(-like) patients.

Besides, immunohistochemistry of desmosomal proteins was performed in myocardial tissue from 3 patients carrying two different *DES* mutations, causing an ARVC(-like) phenotype in one and a severe biventricular cardiomyopathy in two other patients.

Results: No additional *DES* mutations were found in our patient group.

Immunohistochemistry demonstrated accumulation of desmin aggregates with abnormally shaped intercalated disks and normal amounts of desmosomal proteins in p.S13F mutation carriers but a decreased amount of connexin 43, desmoplakin and plakophilin 2 in two patients carrying the p.R454W mutation.

Conclusions: (Probable) ARVC or severe cardiomyopathy with right ventricular involvement are possible phenotypes in DRM. Moreover, we demonstrated that *DES* mutations may affect the architecture and/or the localisation of multiple proteins at the intercalated disk, suggesting a common pathophysiological pathway between arrhythmogenic cardiomyopathies caused by desmosomal gene mutations (mainly affecting the right ventricle) and cardiomyopathies caused by *DES* mutations.

P02.038 The importance of genetic examination in sex assessment in the new born

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Case report: RR, a male infant, 7 months years old, referred in our department, from chirurgery department, for micropenia and facial dimorphism. The child was born at 32 week of gestation, with 1800 g. There was no significant personal or family history for birth defects and genetic disorders. Examinations revealed: weigh 4500gms (< 3th percentile), height of 61 cms (< 3th percentile); dysmorphic facial features (macrocephaly- head circumference of 40 cms, frontal bossing, hypotelorism, prominent eyes, proeminent philtrum), anterior ears, micropenis (0.8 cms), small scrotum and no palpably testis, without associated external genitalia ambiguity. According to the clinical appearance small phallus and scrotum the child get a male sex at birth. After inadequate growth of genitalia the parents decided to have a chirurgery procedure for solve the problem. Laboratory and exams reveals : TSH, Ft3, LH, FSH, Testosterone normal, 17OHP (16.9ng/ml), DHEA (1.57ng/ml). Bone age concordant with chronological age. Ultrashall of the pelvis- a uterus may be present; The hyperplasia of the adrenal glands: 1/0.9/08 mms. MRI - no evidence of the uterus. Cariotype 46XX. The DNA testis for Y chromosome - negative for Y chromosome. In this moment refuse to change the social sex of child.

Conclusions:

1. The clinical appearance of external genitalia may be not sufficient to give the social sex of children.
2. The micropenis in the infant is a real challenge to diagnose and treat.
3. The congenital adrenal hyperplasia with pseudohermafroditism is a rare disease with severe psychological implications for the child and his family.

P02.039 Cerebrofaciothoracic Syndrome: Twin presentation

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Objective: To describe two female dizygotic twins, born to non consanguineous parents by means of assisted fertilization, who had facial dysmorphism, complex anomalies of vertebrae and ribs (such as bifid ribs, hemivertebrae and costal synostosis), and global developmental delay.

Methodology: Presentation of two girls who were studied because of facial and thoracic dysmorphism and mental retardation.

Conclusion and Discussion: The features are similar to those initially described by Pascual-Castroviejo et al in 1975 and thus represent new cases of cerebrofaciothoracic dysplasia (OMIM 213980). Only 13 cases have been previously described. The information obtained through the few cases described so far suggests that most likely this is a hereditary condition transmitted as an autosomal recessive trait. There is a broad spectrum of differences in the phenotypic presentation between the sisters and also compared with the 13 cases previously described), which suggest an autosomal recessive form with wide clinical variability. This may reflect an interaction with other loci, or epigenetic modifications. The interaction with a particular intrauterin factor should be discarded in this case, owing to the twinning state of the sibs. We also describe some anomalies unreported to date in this syndrome such as cardiac malformations, affection of the auditory conduits, umbilical hernia, fifth finger clinodactyly and Arnold Chiari type I. As more patients with this condition are described, the main features of this syndrome are becoming clearer, and also the inheritance pattern.

P02.040 A novel splice mutation in *CYP27A1* associated with cerebrotendinous xanthomatosis (and pulverulent cataract)

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Cerebrotendinous xanthomatosis (CTX) is a lipid storage metabolism disorder, inherited in an autosomal recessive manner. CTX is characterized by neurological findings (such as cerebellar ataxia and dementia), premature atherosclerosis, tendon xanthomas and cataracts. It is caused by mutations in *CYP27A1*, leading to sterol 27-hydroxylase deficiency and abnormal cholestanol tissue deposition.

A consanguineous Bangladeshi family with three children with pulverulent cataract and global developmental delay were ascertained. Since learning difficulties could also be observed in two of the other siblings who showed no evidence of cataract, the underlying diagnosis and inheritance pattern was unclear.

Genetic linkage studies, employing an autozygosity mapping approach, were undertaken to ascertain the underlying genetic cause. The results showed linkage to the chromosomal region 2q35-q36 which contained the *CYP27A1* gene. Direct sequencing of *CYP27A1* revealed a homozygous splice mutation in all family members with cataracts.

This finding confirms the validity of this approach when identifying genes responsible for disorders, even when the aetiology of the condition and diagnosis are uncertain. It highlights the importance of evaluating if CTX is an appropriate diagnosis when the proband has cataracts, especially since the condition can be treated if identified before irreversible brain damage.

P02.041 Clinical and genetic analysis of the *CHD7* gene in Korean patients with CHARGE syndrome

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Background: CHARGE syndrome (CS; OMIM 214800) is a rare autosomal dominant disease associated with coloboma, heart defects, choanal atresia, retarded growth and development, genital abnormalities, and ear anomalies. Mutations in the *CHD7* gene have been suggested to be a genetic background of CS.

Methods: In this study, sequence analysis of the *CHD7* gene was performed in four Korean sporadic CS patients who showed clinical manifestations of typical or atypical CS.

Results: Four novel mutations, three nonsense mutations (Ser705X, Arg1069X, and Trp1534X) and one frameshift mutation (Asn-603ThrsX4), were identified in the *CHD7* gene in the patients. Congenital heart disease, aplasia of the semicircular canal, sensoryneural hearing loss, and developmental delay were observed in all patients; however, choanal atresia was not seen in any of them.

Conclusions: The phenotypic expressions of these Korean patients with CS were different from those of other ethnicities, and allelic heterogeneity was found in the *CHD7* gene in Korean patients with CS. Mutation analysis of the *CHD7* gene should be performed in Korean subjects to rule out the possibility of CS, even when clinical manifestations are not typical of CS.

P02.042** Congenital heart defects in patients with a *CHD7*-mutation

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Loss of function mutations in the *CHD7* gene cause CHARGE-syndrome, an autosomal dominantly inherited combination of multiple congenital malformations, including congenital heart defects (CHDs) in 66-92% of the patients. The pathogenesis and type of CHDs in CHARGE syndrome are still largely unknown. Most malformations in

CHARGE syndrome are related to the neural crest (NC), which is in line with our observation of a high CHD7 expression in isolated mouse progenitor NC cells. Cardiac NC cells are involved in pharyngeal arch and conotruncal defects. We hypothesise that CHDs in CHARGE syndrome are NC-related.

We are collecting detailed clinical information on almost 350 patients with a *CHD7*-mutation detected by our group, and compared their CHDs with 381 non-syndromic CHD-patients collected between 2004 and 2008 by EUROCAT.

The first results show a heart defect in 189 out of 231 CHARGE-patients (82%). Detailed cardiac information is available for 163 of these 189 patients. Of all CHDs 31% were NC-related (e.g. tetralogy of Fallot, right descending aorta), 56% possibly NC-related (e.g. non-specified VSDs) and only 13% were non-NC-related (e.g. muscular and membranous VSDs, hypoplastic left heart). In the control group these figures were 15%, 40% and 45% respectively ($p<0.000$).

These data confirm an important role for the cardiac neural crest in the pathogenesis of CHDs in CHARGE syndrome.

P02.043 Dental abnormalities associated with cherubism

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Cherubism is an abnormal hereditary condition characterized by progressive bilateral swelling of the mandible, especially in children. In some cases of cherubism, the entire jaw swells and the eyes turn up, enhancing the cherubic facial appearance. Frequently, dental abnormalities are associated with cherubism: congenitally missing second and third molars; premature exfoliation of the deciduous teeth and displacement of permanent teeth secondary to the jaw lesions. Mutations in *SH3BP2* gene cause cherubism.

Case report: A 9-year-old girl, youngest of three siblings, was diagnosed with cherubism based on clinical findings, radiological manifestations and molecular genetic testing. There was no family history of similar findings.

Results and conclusions: Clinical examination revealed swellings at angles of mandible, an asymmetrical enlargement of the mandible and chubbiness of the face. Mixed dentition and poor occlusion were noticed. No ocular, respiratory or cardiovascular problems were found. Except her facial appearance, bad alignment of the teeth and malocclusion, the patient did not exhibit any physical abnormality and showed no signs of mental retardation. Panoramic radiograph showed clearly that the facial enlargement is the result of bone changes. Orthopantomogram revealed bilateral multilocular areas of diminished density in the mandible and congenitally missing second and third lower molars. The maxillary dentition was not affected. A mutation was identified in the *SH3BP2* gene in proband and her asymptomatic mother. Lack of signs in mother and the difference in clinical presentation between patient and her carrier mother are consistent with incomplete penetrance in females and variable expression of mutation in *SH3BP2* gene.

P02.044 Familial supernumerary teeth due to cleidocranial dysplasia

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Cleidocranial dysplasia is a rare generalized dysplasia of osseous and dental tissues with an autosomal dominant inheritance. The disorder is characterized by typical facial and dental appearance, skeletal dysplasia and short stature, the two most striking features being hypo or aplastic clavicles and a surprisingly increased number of supernumerary teeth. Intrafamilial variations of the skeletal abnormalities have been described, but those of dental abnormalities are obscure yet. The majority of cases occur due to loss of function mutations in the *RUNX2* gene, which encodes for a transcription factor that is essential for osteoblast differentiation and chondrocyte maturation. It is estimated that the proportion of cases caused by a *de novo* mutation is high. We present the case of a newborn female with skeletal findings consistent with cleidocranial dysplasia, facial dysmorphism with bossing of the forehead, hypertelorism and a depressed nasal bridge, widely open anterior fontanelle, high-arched palate. On lateral and postero-anterior cephalometric radiographs, we could find unclosed coronal suture. Her mother, 31 years old, has short stature, the same dysmorphic features of the face, patent anterior fontanelle, short, broad thumbs, retained

primary teeth. On panoramic radiographic examination unerupted supernumerary teeth in both maxilla and mandible were seen (total of 19). The probands grandmother was also said to have abnormalities in the numbers and eruption of the permanent teeth. In the management of the disease dental procedures, speech therapy, head protection, preventive treatment for osteoporosis are considered.

P02.045 Array-CGH detects mosaic tetrasomy 12p (Pallister-Killian syndrome) in peripheral blood without invasive skin biopsy

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We report on a 3 month old girl presenting with multiple dysmorphic features including hypotonia, irregular respiration, hypertelorism, long philtrum, prominent forehead, retrognathia, low set, dysplastic ears, and impaired hearing. She is the second child of a non consanguineous couple. Karyotyping of peripheral blood revealed an inconspicuous karyotype. Because of the profound psychomotor retardation at the age of six months, Array-CGH-analysis was requested.

Array-CGH with genomic DNA from peripheral blood using 105K oligo arrays, revealed an amplification of the complete short arm of chromosome 12, which led to the assumption of an additional marker chromosome 12 and most probably of a mosaic isochromosome 12p which is common in patients presenting with Pallister-Killian syndrome. Karyotyping of 100 metaphases and interphase FISH analysis on nuclei from cultured T-lymphocytes using a chromosome 12 centromeric probe failed, simply because detection of an isochromosome 12p in stimulated T-lymphocytes is hard to achieve as only 1-2% of lymphocyte metaphases contain the additional isochromosome 12p.

The subsequent karyotyping on fibroblasts of a cultured skin biopsy confirmed the presence of an isochromosome 12p (tetrasomy 12p) in 56% of cells. These results refer to Pallister-Killian syndrome and the girl reported shows several correlating features.

Up to now, analysis of fibroblasts from invasive skin biopsies was the only opportunity to detect the additional isochromosome 12p in Pallister-Killian syndrome patients. Array-CGH with genomic DNA from peripheral blood, however, is an adequate and powerful tool to investigate children suspected of having Pallister-Killian syndrome. An invasive skin biopsy is no longer required.

P02.046 High-Level expression of functional recombinant human coagulation factor VII in insect cells

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Recombinant coagulation factor VII (FVII) has been introduced as a potential therapeutic intervention in hemophilia patients who produce antibodies against the coagulation factors. Mammalian cell lines provide only low levels of expression, however the *Spodoptera frugiperda* cell line Sf9 and baculovirus have proved to be a powerful system for high-level expression of recombinant proteins. Due to the lack of endogenous vitamin K-dependent carboxylase, expression of functional FVII through this system is impossible. In the present study, we report a simple but versatile method to overcome the defect for high-level expression of the functional recombinant coagulation FVII in Sf9 cells. This method involves expression of both the human gamma-carboxylase (hGC) and human FVII genes in the host. The cDNAs of hGC and FVII were isolated and cloned into appropriate vectors. Recombinant baculoviruses carrying the human FVII and hGC genes were generated and insect cells were directly coinfecte with the recombinant baculoviruses. Expression of the recombinant FVII (rFVII) was verified, purified, and the biological activity was determined. The expression of FVII and hGC mRNAs in coinfecte Sf9 cells were detected by RT-PCR and further confirmed by Western blot analysis. A fourfold decrease in clotting time was observed. Whereas no decrease in prothrombin time was noted when the rFVII expressed in the absence of hGC, the results indicate that the expression of hGC confers functional activity of rFVII. Therefore it may possible to express other vitamin K-dependent coagulation factors using this method in the future.

P02.047 Ophthalmic findings in the Greek isolate of Cohen syndrome.

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Cohen syndrome is a rare condition of mild to moderate developmental delay, characteristic craniofacial features, childhood hypotonia, joint hyperextensibility, neutropenia, and a variety of ophthalmic abnormalities. A high frequency of the syndrome has been observed in a Greek island with 2,000 inhabitants and a high degree of inbreeding. All patients were homozygous for a *COH1* deletion of exons 6 to 16, suggesting a founder effect. We present the results of their first systematic ophthalmologic assessment.

Myopia and chorioretinal atrophy were present in all patients of this cohort. Yet, in contrast to all groups previously reported, the majority presented corneal changes, independently from age, gender and family history. A pair of sisters, aged 11 and 15 years old, presented bilateral keratoconus. More frequently (86%) than in any other ethnic group, Greek patients presented cataracts that were bilateral and often graded as high as 3, even at a young age. As a whole, the ophthalmic phenotype of the Greek isolate of Cohen syndrome was characterized by the involvement of both the posterior and the anterior eye segment, bilaterally, in the majority of cases (93%).

Greek Cohen subjects that share a founder mutation are at a higher risk of developing blindness in respect to those of other ethnicities and genotypes. This study added to the range of visual problems seen in children and adults with Cohen syndrome the finding of thin corneas and highlighted the need for pachymetry measurement as a means of surveillance and prediction of the visual impairment frequently observed.

P02.048 Two years experience of *VPS13B* gene sequencing: confirmation of diagnostic criteria, value of array-CGH in identifying large rearrangements and identification of a new phenotype

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Cohen syndrome is a rare autosomal recessive inherited disorder that results from mutations in the *VPS13B* gene. Clinical features consist of a combination of mental retardation, facial dysmorphism, post-natal microcephaly, truncal obesity, slender extremities, joint hyperextensibility, myopia, progressive chorioretinal dystrophy and intermittent neutropenia. From the experience of 42 patients (36 families) referred for molecular analysis of *VPS13B* for suspicion of Cohen syndrome, two mutations were found in 7 families, one mutation was found in 2 families, and none in 27 families. 244K array-CGH revealed two intragenic rearrangements in the two families with only one mutation (one deletion and one duplication). We found that the presence of chorioretinal dystrophy (92% versus 32%, p=0.0023), intermittent neutropenia (92% versus 5%, p<0.001) and postnatal microcephaly (100% versus 48%, p=0.0045) was significantly higher in the group of patients with *VPS13B* gene mutations compared to the group of patients without mutations. All patients with *VPS13B* mutations had chorioretinal dystrophy and/or intermittent neutropenia. The Kolehmainen diagnostic criteria provided 100% sensitivity and 77% specificity. Interestingly, we found an adult aged 34 years compound heterozygous for two splicing variants (IVS34+2T>+3AinsT and IVS57+2T>C) with dysmorphic features, normal incisors, microcephaly, severe neutropenia responsible of recurrent infections at age 2 years and chorioretinal dystrophy at age 22 years but absence of obesity and mental retardation (total IQ = 100). This observation enlarges the phenotype of the *VPS13B* gene, but further underscores the importance of the two key features in the indication for a *VPS13B* gene study.

P02.049 P628L and G624D *COL4A5* mutations do not cause classic X-linked Alport Syndrome but a milder nephropathy resembling Thin Basement Membrane Nephropathy

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Classical X-linked Alport syndrome (XLAS), caused by mutations in *COL4A5*, is associated with childhood hematuria progressing to proteinuria, chronic renal failure (CRF) and end-stage kidney disease (ESKD), often accompanied by deafness. Renal biopsies present splitting, lamellation and thickening of the glomerular basement membrane.

We studied clinically and genetically two Greek and two Cypriot families with a clinical and pedigree based suspicion of XLAS despite presenting mild characteristics of the syndrome. DNA linkage analysis and direct sequencing resulted in the identification of mutation *COL4A5*-G624D in the Greek families and *COL4A5*-P628L mutation in the Cypriot families. The clinical characteristics of most male patients in these four families are surprisingly mild. Four males currying G624D reached ESKD after the age of 39 and one showed Thin Basement Membrane Nephropathy (TBMN, a mild nephropathy caused in many cases by heterozygous *COL4A3* or *COL4A4* mutations) on Electron Microscope. Another five of nine males with mild mutation P628L developed ESKD between 30 and 57 years while three show mild CRF at similar ages. Biopsy in one of them showed TBMN. Mutations G624D and P628L are very near the 12th natural interruption of the *COL4A5* triple helical domain and this may explain the much milder phenotype. Concluding, some *COL4A5* mutations may not lead to ESKD until the 40's and 50's. The phenotypic spectrum may extend to milder forms such as TBMN and late-onset ESKD. This wider or milder spectrum of symptoms may be attributed to the position of the mutation relative to the collagenic interruptions.

P02.050 Familial congenital diaphragmatic hernia, cystic kidneys and cardiac anomalies: A new X-linked syndrome?

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Congenital diaphragmatic hernia (CDH) occurs in approximately 1 in 3000 live births and is associated with significant morbidity and mortality. Etiologically, CDH is a heterogeneous with most isolated cases being multifactorial. About 50% of the cases are 'non-isolated', with associated extra-pulmonary anomalies. Some of these cases are single gene disorders and 5-10% have a chromosomal aetiology.

We describe two male siblings of non-consanguineous Caucasian parents with CDH, distinctive facial dysmorphism, cardiac anomalies and renal cysts - a constellation of features not consistent with other previously described CDH syndromes. Both siblings exhibited coarse facial features, hypertelorism, brachydactyly, dilatation of the ascending aorta, valvular dysplasia and enlarged cystic kidneys. In addition, sibling two had hepatomegaly, splenomegaly and enlarged thymus as well some intracranial white matter calcification. Karyotype in both siblings was 46, XY and microarray analysis in the second sib was normal. A maternal uncle had died in infancy as the result of CDH. Thus, the pedigree is strongly suggestive of X-linked inheritance.

We review the features of other known X-linked CDH syndromes which include Simpson-Golabi-Behmel syndrome, Craniofrontonasal syndrome, Thoracolumbar syndrome and X-linked Cornelia De Lange syndrome. None of these are reconciled by the phenotype in our two cases and indeed, genetic testing for *GPC3* to rule out Simpson-Golabi-Behmel syndrome was negative. We believe that this family exhibits a new X-linked genetic syndrome of which congenital diaphragmatic hernia is a principal feature.

P02.051 Molecular karyotyping provides etiological diagnosis in two malformative patients with blurred chromosomal aberrations

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Array CGH has proved useful as a diagnostic tool for patients with developmental delay and congenital malformations. We screened 2 patients with ambiguous cytogenetic results in order to disclosure the molecular basis of the mutations. We have used genomic array Cyto-Chip (BlueGnome, Cambridge, UK), covering the entire genome at a median 565Kb. It investigate sub-telomeres at a median 250Kb resolution, reliably detect mosaicism and examine 90 known genetic conditions at a median 100Kb resolution.

Case 1. 10 years old girl with Kabuki make-up syndrome characterized by craniofacial dysmorphism: hypertelorism, up slanting palpebral fissures, ptosis, short philtrum, depressed nasal bridge, microretrognathia, preauricular tag; generalized hypotonia, bilateral simian creases, wide spaced nipples, four toes clinodactyly of the toes. The karyotype was 46,XX,add(4)(q35) and array CGH revealed a duplication of (6)(q25.1;q25.3) spanning 6.377Mb as well as a 4q35.1 duplication of 3.815Mb.

Case 2. In the second case with karyotype 46,XX,add(17)(q25) array CGH detected duplication of (17)(q24.2;q25.1) region encompassing 9.58Mb in a 1 year old girl with polydactyly of toes and fingers and craniofacial dysmorphism: microcephaly, hypertelorism, low-set ears, high palate.

Confirmatory FISH studies with BAC clones are planned for accurate confirmation of CytoChip result. The influence of the known genes in the imbalanced regions and their correlation to the phenotype will be discussed.

In conclusion, we demonstrate that molecular karyotyping enables a diagnosis in patients with unclear cytogenetic results. Furthermore, we demonstrate the effect of causal CNVs on the development of dysmorphism.

P02.052 Associated malformations in patients with limb reduction deficiencies

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Infants with limb reduction deficiencies (LRD) often have other associated congenital malformations. The purpose of this investigation was to assess the prevalence and the types of associated malformations in a defined population. This study included special strengths: each affected child was examined by a geneticist, all elective terminations were ascertained, and the surveillance for malformations was continued until 1 year of age. The associated malformations in infants with LRD were collected in all livebirths, stillbirths and terminations of pregnancy during 26 years in 347,810 consecutive births in the area covered by our population based registry of congenital malformations. Of the 271 LRD infants born during this period, representing a prevalence of 7.8 per 10,000, 57.9% had associated malformations. There were 17(6.3%) patients with chromosomal abnormalities including 10 trisomies 18, and 62 (22.9%) nonchromosomal recognized dysmorphic conditions. There were no predominant recognised dysmorphic conditions, but VA(C)TER(L) association. However numerous recognised dysmorphic conditions were registered. Seventy eight (28.8 %) of the patients were multiply, non syndromic, non chromosomal malformed infants (MCA). Malformations in the urogenital system (n=50, 14.2%), the cardiovascular system (n=40, 11.4%), the central nervous system (n=27, 7.7%), and the digestive system (n=16, 4.6%) were the most common other malformations. The prevalence of associated malformations, which was more than one in two infants, emphasizes the need for a thorough investigation of infants with LRD. A routine screening for other malformations especially cardiovascular system, urogenital system, central nervous system, and digestive system may be considered in infants and in fetuses with LRD.

P02.053 Further case of 12q duplication: Wolf- Hirschhorn Syndrome phenocopy or distinct syndrome?

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Interstitial duplication of short arm of chromosome 12 is a very rare cytogenetic syndrome previously reported as a phenocopy of Wolf-Hirschhorn Syndrome (DallaPiccola et al., 2008).

We present a new case of 12q13 duplication in a 6 years old girl with similar dysmorphic features in early infancy, but with a facial phenotype evolving with age and suggesting a possible different gestalt in older patients.

She was born at 31weeks of gestation after uneventful pregnancy. Examination in neonatal period showed measurements under 3rd centile, palatoschisis, lacrimal duct obstruction, malar hypoplasia, shallow orbit with exophthalmos, hypertelorism, down-slanting palpebral fissures, high arched eyebrows, cleft palate, corneal opacity.

Early motor development was slightly delayed. At the age of 2 some similarities with Wolf- Hirschhorn Syndrome dysmorphic features were noted.

At 6 years she presents with an ataxic but autonomous walk and a border line - mild mental retardation. In addition to previous dysmorphic features, she has a longer face, prominent nasal bridge, and prominent superior-orbit ridge. Hands are small and show a thick, slightly redundant and hyperkeratinized skin.

CGH Array analysis (CytoChipOligo ISCA 4X44K) with a resolution of 75Kb, revealed a duplication of the long arm of chromosome 12 (dup12q13.13).

Compared to the previous case, our patient carries a smaller duplication. Although some features of the present patient at birth and at the age of 2 are reminiscent of the Wolf-Hirschhorn Syndrome, the actual clinical picture appears different.

Differences with the previously reported case will be discussed in terms of age and of size of duplication region.

P02.054 Prenatal diagnosis of Apert syndrome-case report

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Apert syndrome is one of the craniosynostosis syndromes. It has an autosomal dominant inheritance and is characterized by the association of craniostenosis and osseous and membranous syndactyly of the four extremities. The incidence has been estimated at 1/50000 births. The craniosynostosis is bicoronal and is present at birth. Most of the cases are "de novo" mutations, and could be associated with advanced paternal age.

We present a case of pregnant woman who came for second opinion of ultrasound investigation for severe anomalies of extremities, detected at 21 weeks of pregnancy. Those was characterized by complete syndactyly of all extremities, with absence of movements and also associated anomaly of cranian shape, bilateral cleft lip and palate. Those dates rised the suspicion of Apert syndrome. It is worth mentioning advance paternal age of 55 years old. Because of the severity of anomalies detected the family decided to interrupt the pregnancy and clinical examination of the fetus confirm the diagnosis of Apert syndrome.

P02.055 First cases of Craniosynostosis diagnosed by custom molecular microarray (Array CGC)

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Introduction: Craniosynostosis is a condition of variable aetiology characterized by premature closure of calvarial skull bones. With many sutures closing prematurely, the skull cannot expand to accommodate

the growing brain, which leads to several consequences, including developmental delay, mental retardation and vision and hearing problems can also appear. Most cases are diagnosed during the neonatal period but many cases are detected sooner by the ultrasound during the prenatal period. Because this disorder is clinically heterogeneous, some carriers are not detected until late in infancy or only detected after the birth of an affected child. The major conditions, Muenke, Pfeiffer, Apert, Crouzon, Jackson-Weiss, and Seathre-Chotzen-like Syndromes, are related with mutations located in the different genes of the Fibroblast Growth Factor Receptors (FGFR) 1, 2 and 3.

Method: Using new custom microarray panel (Arrays CGC - Patent Pending) that contains a panel of 10 point mutations, identified in 4 main genes involved on syndromic craniosynostosis it is possible to identify the molecular basis of the most frequent and severe forms. We report here our experience using this new methodology.

Results: We analyzed 9 cases (8 peripheral blood and 1 amniotic fluid) and in two we detected the c.866A>C (p.Gln289Pro) mutation in heterozygosity in the FGFR2 gene and the c.755C>G (p.Ser252Trp) in the FGFR2 gene. Turnaround time was 2 weeks.

Conclusion: This approach drastically reduces turnaround time, maintaining accuracy and liability. Faster diagnostic is achieved, allowing early decision-making process in patient management.

P02.056 Craniostenosis, pigmentary disorder and hemimegalencephaly in a patient with fibroblast growth factor receptor 3 (FGFR3) mutation.

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We describe a 2-year-old Egyptian boy, born with abnormal skull shape to healthy consanguineous young parents. At 2 months he showed: midface hypoplasia, proptosis, and low-set ears, not associated with acanthosis nigricans. He had several oval hypomelanotic patches on the skin. Mutation analysis of exons IIIa and IIIc in *FGFR2* and *FGFR3* and screening of the two genes of *TSC* mutations were performed. A 3D CT scan of the cranium showed synostosis of the uni-coronal, sagittal and lambdoid sutures, whereas brain MRI showed left hemimegalencephaly, hypoplasia of the corpus callosum and abnormal hippocampus. The patient was found to have a de novo point mutation of the *FGFR3* gene. A major screening of the two genes of *TSC* mutations was negative. At 18 months of age he developed obstructive hydrocephalus. His development is normal and his neurological examination showed no asymmetry of movements, tone and reflexes. Apart from febrile seizures, there is no history of epilepsy until now. We think this is a previously unreported association which could be a new syndrome allelic to Crouzon dermoskeletal syndrome. There is phenotypical overlap with Muenke syndrome and craniofacial dysostosis. Hydrocephalus should be detected early for opportune treatment.

P02.057 Longer term survival of a child with autosomal recessive cutis laxa due to a novel FIBULIN 4 mutation.

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Autosomal recessive cutis laxa (ARCL) is a heterogeneous connective tissue disorder which continues to be further sub classified by molecular etiology. We describe a six year old boy who presented with severe aortic root dilatation at one year of age requiring semi urgent surgical repair. He also has arterial tortuosity, generalized hypermobility, mild cutis laxa and a history of bilateral inguinal hernias. His parents are consanguineous and related to the parents of three distant cousins who all died in the first few years of life. These children were originally published by Welsh et al in 1971 as a novel connective tissue disorder. We identified candidate regions using homozygosity mapping and a high density SNP array; genotyping the obligate carrier parents of

the deceased children and our patient. An inspection of the genes in these regions identified Fibulin 4 (EFEMP2) as a likely candidate, and a homozygous nonsynonymous mutation at c.376 G>A (p.E126K) in exon 4 was identified in the patient. The obligate carriers were heterozygous at this position, and the mutation was not present in healthy controls. All amino acid changes at this highly conserved region in the EGF binding domain are predicted to be damaging. This is only the fourth family, to our knowledge, with ARCL due to Fibulin 4 deficiency and the emerging phenotype of this subgroup of patients will be reviewed. Although postulated in the literature to be universally lethal, our experience suggests that early and aggressive treatment of aortic aneurysms can result in longer term survival.

P02.058 Trisomy 21 and false positive sweat test

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The paper evaluates the possible relation of 21 trisomy and sweat test result in cystic fibrosis. Material and method: 5 month old girl infant was admitted to hospital for evaluation of a particular phenotype. The clinical examination at admission revealed hypotrophic child with particular phenotype (epicanthic skin folds, eyelid slit, low-seat ears, hypertelorism, epicanthus, a slightly open mouth with tongue protrusion, simian crease, a groove between the great toe and the second toe = the sandal sign), pallor, globally reduced tissue, a hypotonia, no pathological change in lungs at auscultation, systolic murmur of III /VI degrees well-beating peripheral pulse, abdominal hypotonia with right abdominal diastasis, liver and spleen within normal limits. The particular phenotype was assimilated to a Langdon-Down syndrome Results: The karyotype has confirmed a structural chromosomal abnormality of robertsonian translocation type between acrocentric chromosomes 21 and 22, and a numerical chromosomal abnormality consistent with a total trisomy 21 type, the cytopenic formula being: 46, XX, -22, +21, tris (21;22). The sweat test was positive; NaCl 86 mmol/l and 98 mmol/l the second test. The genetic analysis for cystic fibrosis was negative for the 29 most common mutations for central-eastern european area. Echocardiography revealed a common atrioventricular canal complete form. An abdominal ultrasonography showed several images interpreted as cholelithiasis. Conclusion: The question is if there is an association between trisomy 21 and cystic fibrosis or another condition is the leading cause of false positive sweat test still has to be answered.

P02.059 Trends in Median Age at Death from Cystic Fibrosis in Brazil from 1979 to 2005

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The aim of this work is to display the temporal trends in median age at death (MAD) from cystic fibrosis (CF) in Brazil, and to compare these findings with other international trends in CF MAD. Fogarty et al (2000) investigating the same parameter for developed countries from 1974 to 1994, came to the conclusion that MAD from CF was increasing, but their data implied that clinically significant differences in survival with CF persist between countries. Therefore, we investigated all persons registered in Brazil as having died of CF in specified years. Mortality data for CF (International Classification Disease CID- 9 code 277.0 between 1979 and 1995; and CID-10 codes E84.0; E84.1; E84.8 and E84.9 between 1996 and 2005) were obtained from the Brazilian System for Mortality Information - SIM Data SUS. In Brazil, the total number of notified deaths from CF was 1455 from 1979 to 2005. We found that, in 1979, the MAD was 3.57 years, and, in 2005, it was 23.45 years. The Brazilian means are much lower than those found by Fogarty and collaborators for developed countries. In 1994, they found a MAD of 21 y. and in Brazil it was 12, 5 y. In 1979, the MAD for our country was 3,39 y, while five years earlier, in 1974, they found a MAD of 8 y for developed countries. Furthermore, our results have shown that no increase of MAD occurred in Brazil from 1979 to 1984, however from 1984 to 2005 it had a sixfold increase.

P02.060 De novo interstitial 8p23.1 deletions identified by array-CGH in two patients: definition of the critical region for cardiac malformation.

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8p23.1 deletions have recently been described as cause of complex congenital heart defects and diaphragmatic hernias. Moreover haploinsufficiency of GATA4, located at 8p23.1, is thought to play a critical role in the cardiac development.

An interstitial deletion of 8p23.1 has been reported only in 9 children; in 4 of them it has been characterized using array-CGH analysis, while in the remaining 5 cases it was detected by FISH analysis. All cases reported to date are *de novo*.

We describe two unrelated patients with complex congenital heart malformation, microcephaly, plagioccephaly and similar face dysmorphisms. Array-CGH analysis (Agilent platform, 44K) revealed a microdeletion of 8p23.1 in both patients (3.5 Mb and 4.9 Mb in size, respectively).

In the 6 children investigated by array-CGH (our 2 patients and the 4 cases in literature) we could identify a common region spanning from 8,850,913 bp to 11,770,357 bp. GATA4 is the only gene in this deleted region with a suggested role in heart development and more studies are needed to clarify its involvement in determining the cardiac phenotype.

Our observation further stresses the importance of performing array-CGH in children with complex heart malformations and minor dysmorphisms, as it is possible that other genes mapping within the deleted region might contribute to the described characteristic phenotype of del8p23.1 syndrome.

Investigating the precise size of the deleted region is mandatory to delineate the critical region of the del8p23.1 syndrome.

P02.061 Deletion 3p syndrome and phenotypic change with age: an adult case report

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Introduction: The deletion of the short arm of the chromosome 3 is a rare disorder characterized by growth failure, psychomotor and mental retardation and craniofacial anomalies and less commonly others cardiac, renal and gastrointestinal malformations.

Case report: The propositus is male reported at the age of 20 months for the presence of congenital malformation and hypotonia. The karyotype was 46,XY,del(3p26). Currently he is 18 years old and exhibits evidence of phenotype changes no characteristics of the syndrome at pediatric ages. Physical examination revealed a height of 167 cm, weight of 47 kg. He is normocephalic with triangular face, malformed ears, low frontal hairline; low nasal base and bridge, absent and long philtrum, full inferior lip, narrow and high arched palate; hypotrophic limbs, hands with cutaneous syndactyly and bilateral clinodactyly in the fifth finger. Neurological examination is normal.

Discussion: The deletion 3p syndrome it has a characteristic phenotype, nevertheless a few adult cases were reported. The patient shows some physical findings not compatible with the syndrome and could be age-related. Other are more associated with the extension of the deletion.

Conclusion: This syndrome presents a strong connection between the severity of the disease and the portion of the deletion. The previous statement justifies the importance of delimit the affected chromosomal area with molecular characterization and the phenotype-genotype correlation. We also consider the existence of a phenotypic variability that is related with the age in this syndrome.

P02.062 15.9 Mb terminal deletion in 9p24.3-p22.3: Clinical description of an adult patient with deletion 9p syndrome

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Descriptions of adult patients are rare in the literature. As a consequence, the natural history of many chromosomal disorders is largely unknown, which hampers adequate genetic counselling and keeps parents in the dark about the problems their children may be faced with in the future. Therefore it is important to describe older patients whenever possible, so that informations about the prognosis and course can be provided for the families.

Here we report a 23 year-old women with deletion 9p syndrome. She was of short stature with microcephaly and showed narrow fingers and feet. The major problems in this patient were a marked scoliosis which was operated at the age of 9 years, chewing problems because of micrognathia which was operated at the age of 22 years, pain after walking because of pressures sores on her narrow feet and aggressive behaviour in unfamiliar crowded places. There was precocious puberty with menses occurring at the age of 10 years. Because of regular menstruation she underwent tubal ligation. After the age of about 10 years there were no more incontinence problems. The young lady was able to communicate and to speak in simple words, but not to read or write. She liked very much drawing and listening to music and was described as a very social and usually friendly person.

P02.063 Dento-maxillary abnormalities in Prader Willi syndrome

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Prader Willi Syndrome (PWS) is a rare genetic disease, characterized by multi-systemic damage, predominantly neuro-hormonal. The main clinical features are hypotonia in the newborn, and feeding difficulties later in childhood, hyperphagia and obesity, small stature, small hands and feet, hypogonadism, variable mental retardation, behavioral problems. Clinical characteristics (viscous saliva in small quantity, dry mouth, small jaw, dental anomalies various) make in the current measures of care, to require and dental.

In a multicentric project started in Romania universities, hospital and Prader Willi Association of Romania, the study of oro-dento-maxillary issues revealed some interesting aspects. In all 8 cases during 2009 followed PWS were identified serious dental problems. 5 of the patients were children (5-12 years) and 3 were adults (22-28 years). In 7 cases (87.5%) were identified caries and bacteriological investigations has highlighted the presence of germs over limit. One of the patients had a Class III Angle malocclusion, maxilar hypoplasia and severe deficiency of enamel. Dental examination and discussions with parents revealed deficiencies in preventative measures: first oral examination when teeth eruption is temporary, carefully supervised oral hygiene parents or guardians, the use of topical fluoride, regular checks and dental treatment, if necessary. These flaws are due to be capacity limited collaboration of patients or lack indications for periodicals dental examination.

Because of the complexity of medical issues present in these patients, the study reveals the importance of the participation of multidisciplinary teams that can provide an integrate treatment. Issues brought before opening new areas for further study.

P02.064 Functional impact of transitional developmental defects of the lumbar spine

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The restructuring process of the spine for adaptation to the new mechanical conditions imposed by biped position required complex structural changes. There are individual predisposing factors with important contributions to the changes of the spine: phylogenetic variations of cranial or caudal spine development, spina bifida occulta, spondylolisthesis and spondylolysis. Variations in the number, position, shape of the vertebrae are common developmental anomalies. A genetic predisposition is believed to be involved, as for some of these, family members have incidences of 28-69%. The aim of the current study was to reveal the presence of the lumbar vertebral anomalies and their functional impact in patients with low back pain treated in the Rehabilitation Clinical Hospital Baile Felix. Out of 262 examined cases, 32.82

% had vertebral anomalies, 42 women and 44 men, with an average age of 29.82 ± 3.42 years. To highlight the impact of low back pain due to vertebral anomalies on functionality and daily activities we used Oswestry questionnaire. Average score was $33.79 \pm 13.21\%$, revealing a moderate disability. Oswestry score revealed loss of 40% for good physical condition, lifting weights, and walking. A deficit of 44.28% was noticed for standing, 30% for social life and 27.14% for professional life. The majority of congenital transitional vertebral anomalies pose no problems for the patient during childhood and thus go undetected, but they may be the cause of low back pain. Physical therapy, therapeutic tool of choice, is able to solve several issues related to the functional impact of these defects.

P02.065 Survey of DFNB59 gene mutations in exon 2 association with deafness children in Kohkeloie va BoerAhmad s. Ghasemi, m. taherzadeh, E. Farokhi, M. Hashemzadeh;

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Introduction: Hearing loss is a heterogeneous disorder and may be due to genetic or environmental cause. PEJVAKIN (DFNB59) a newly identified gene on chromosome 2q31.1-q31.3 encodes a 325-residue protein. This gene mutated in some deaf people with autosomal recessive heredity. This study aims to determine the frequency of the PEJVAKIN gene mutations in 88 deaf children in Kohkeloie va BoerAhmad province. This gene was surveyed with PCR-SSCP method. SSCP is defined as conformational difference of single stranded nucleotide.

Methods and materials: In this descriptive-lab based study, the blood sampling for these children was done. Then, DNA was extracted from all patients following the standard phenol-chloroform produce. Then, the fragment was amplified by Touch-down PCR. So, denaturing buffer was added to PCR products, and then was taken in boiling water. Finality, the single stranded was surveyed by 6% polyacrilamid gel.

Results: Based on data from the present study, was concluded that DFNB59 gene mutations in exon 2 have not contribution in deaf children in Kohkeloie va boerAhmad province.

P02.066 DK phocomelia syndrome with thrombocytopenia, encephalocele and choanal atresia in an adult male with moderate learning difficulties

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We report a 29 year old male, second child of unrelated parents, who was born at term by normal delivery with bilateral choanal atresia, a posterior encephalocele, and bilateral upper limb defects involving both radial and ulnar rays. He was treated for a left convergent squint as a baby by patching, and he was found to have moderate global learning difficulties. His height is on the 0.4th centile and his head circumference on the 97th centile. His platelet count was below the normal range on two occasions. He had surgery for undescended testes aged 12 years, and he was commenced on androgen replacement therapy for hypogonadism aged 27 years. He has a severe thoracolumbar scoliosis with dorsal kyphosis. We propose a diagnosis of DK phocomelia syndrome.

P02.067 Prognostic insights in subjects with subclinical hypothyroidism and Down syndrome

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Introduction: Down syndrome (DS) is associated with thyroid disorders including hyperthyroidism, overt hypothyroidism (OH) and subclinical hypothyroidism (SH; $5 < \text{TSH} < 10 \text{ mU/L}$, normal free thyroxine). In DS with SH, there is a controversy regarding the frequency of thyroid testing, recommended every 3-12 months, and the identification of autoimmunity as a prognostic factor.

Methods: 240 DS subjects (130M, 110F; 1 month-21 years) were evaluated at the Federico II University, Department of Pediatrics, Naples. Thyroid function was tested yearly; in case of $5 < \text{TSH} < 10 \text{ mU/L}$, TSH, FT_3 , FT_4 , anti-TG and anti-TPO were screened quarterly. In case of $\text{TSH} > 10$, the test was repeated prior to thyroxine therapy.

Results: The prevalence of thyroid disorders was 65.4%: 73.8% of patients had SH, 24.2% OH and 1.9% hyperthyroidism. Anti-TPO and

anti-TG were present in 16.6% of cases. The rate of thyroid antibodies was 7.5% in SH and 7.1% in OH. The time-course between the first finding of hyperthyrotropinemia and $\text{TSH} > 10$ ranged from 1 to 13.8 years: 33.3% of patients reached $\text{TSH} > 10$ after 11.6-24 months, 14.9% after 24-36 months and 51.7% over 36 months.

Autoantibodies were present in 13.3%, 23% and 17.7% of cases of the first, the second and the third group, respectively. In 4.4% of cases, $\text{TSH} > 10$ spontaneously normalized without therapy. The prevalence of SH was independent from age.

Conclusion: The prevalence of SH in DS is high. The occurrence of SH is age-independent. Autoimmunity is not a prognostic factor for SH or OH. Because of the different time-course of TSH levels, blood screening can be carried-out every 12 months.

P02.068 Clinical diagnosis problems - Cockayne or Dubowitz syndrome?

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We present a 12 years 5 months old girl with postnatal growth retardation, microcephaly, peculiar facial dysmorphism, mental retardation and recidivist eczema on legs and hands (first time appeared at 8 years age). She was born un term after an uncomplicated pregnancy as the first child of a healthy, nonconsanguineous couple. Initially she was diagnosis with observation. Cockayne syndrome and fellow up attentively (but neurologic dysfunction are not progressive). Based an phenotypic features she was diagnosis with Dubowitz syndrome.

The Dubowitz syndrome is a rare, autosomal, recessively inherited disorder of intrauterine and postnatal growth retardation leading to microcephaly, moderate mental retardation and such characteristic facial anomalies as telecanthus, epicanthic folds, blepharophimosis, ptosis, broadening of the bridge and tip of the nose, abnormal ears and retrogenia. Further findings include hyperactivity, eczema and brachyclinodactyly of the fifth fingers.

Proband' karyotype was normal. As a particularity the proband presented hypercholesterolemia.

The diagnostic symptoms and the differential diagnosis are discussed.

P02.069 Prenatally diagnosed siblings with dysgnathia complex

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Dysgnathia complex is an extremely rare multiple congenital anomaly syndrome. It is characterized by severe mandibular hypoplasia or agenesis, ear anomalies, microstomia, and microglossia. It is thought to have an autosomal recessive etiology.

We describe a newborn with dysgnathia complex born to a second degree cousin marriage. He was found to have hypoplastic mandible and ear migration anomalies at prenatal ultrasonography. He died an hour after birth.

The first pregnancy of the family had resulted in an unembryonic pregnancy. Second pregnancy had ended in a missed abortion. The third pregnancy was diagnosed as having otocephaly in the second trimester. During the follow up of the fourth pregnancy, antenatal ultrasonography showed similar clinical findings. The infant was born and deceased during the first hour of life.

We will discuss the ultrasonographic and postnatal features of the siblings in comparison with previous patients in the literature. This family is a further evidence for the autosomal recessive etiology of dysgnathia complex.

P02.070 A de novo partial trisomy of distal 6p in patient with severe dysmorphic features

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We report on an eight-year-old boy with chromosome 6 unbalanced aberration and severe dysmorphic features. Our patient is the first

child of the first complicated pregnancy from non-consanguineous parents. The boy's phenotype is characterised by brachycephaly, round face, unilateral ptosis of upper eyelid, short, broad nose with flat nasal tip, long flat philtrum, thin vermillion of upper lip, high arched palate, microstomia, large and prominent pinnae, short neck, several *café au lait* spots on the abdomen, waist and right thigh, hypertrichosis on the back, pilonidal sinus, psychomotor retardation and growth delay. Clinical follow-up showed these clinical findings: Xray showed thymus hyperplasia, heart ultrasound - atrial septal defect and recurrent respiratory infections.

Conventional G-banded karyotype analysis from GTG banded metaphases revealed the primary karyotype of 46, XY, der(6)add(6)(q24) in all cells. Chromosomal analyses of the proband's parents showed normal karyotypes, indicating that the above abnormality was de novo. In order to identify the origin of additional material, FISH analysis using subtelomeric specific probes for all the long and short arm of chromosome was performed. FISH analysis revealed duplication of the 6p subtelomeric region to the 6q subtelomere region and deletion of 6q subtelomeric region.

We make a suggestion that the derivative 6 chromosome comprised due to pericentric inversion which occurred from aberrant homologous recombination in the germline of a parent (germline mosaicism) or shortly after fertilization in the proband cells.

P02.071 DYSCERNE: Developing Clinical Management Guidelines for Selected Dysmorphic Syndromes

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The DYSCERNE Network of Centres of Expertise for dysmorphology (www.dyscerne.org), is developing clinical management guidelines for Williams (WS), Angelman (AS), Noonan (NS) and Kabuki (KS) syndromes. An initial scoping exercise identified these conditions as rare, complex, multi-system disorders, for which it was felt that affected patients, families and health/social care professionals would benefit from access to up-to-date evidence based management guidelines. Published evidence from which to develop management recommendations for these conditions is very limited, and devising a systematic and robust methodology has been challenging. Our approach is based on the Scottish Intercollegiate Guidelines Network (SIGN) method, which we modified placing more emphasis on expert opinion and consensus, whilst maintaining systematic rigour and transparency of processes. The development process includes:

- Identification of key management issues by guideline group leaders.
- Targeted, systematic literature searches using PubMed.
- Review, identification and grading of results by panel of invited experts.
- Consensus meetings at which experts present, discuss and agree recommendations.
- Initial drafting of guideline document which is circulated amongst experts and stakeholders for comments.
- Amendments incorporated and guideline finalised.
- Guidelines piloted and evaluated.
- International dissemination.

The process has involved 49 experts from 8 countries reviewing between them, over 1000 papers. The WS guidelines are currently being piloted in 20 centres, by paediatricians and geneticists. The first draft of AS guidelines has been circulated for feedback. Consensus meetings for NS and KS will be held very shortly. The finished guidelines will be available from the DYSCERNE website.

P02.072 DYSCERNE: Results from the full European launch of the Electronic Dysmorphology Diagnostic System (DDS)

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The rarity of dysmorphic conditions means that even in Centres of Expertise experience may be limited resulting in delayed or uncertain diagnosis. Correct diagnosis is the cornerstone of patient management, enabling clinicians to locate other patients with the same condition, share clinical experience and increase individual and collective knowledge.

The web-based Dysmorphology Diagnostic System (DDS) developed by DYSCERNE (www.dyscerne.org) links thirty-two European Centres of Expertise for Dysmorphology to form a powerful diagnostic resource for rare dysmorphic conditions.

Eighty-five submitting nodes are licensed to submit patients' clinical data and images via an online case submission form, which is then reviewed by thirty-seven Expert Dysmorphologists.

Results since the full launch of the DDS show the number of case submissions and expert reviews per case have steadily increased throughout the year. Summary diagnostic reports are prepared from the consensus of expert opinions and sent to the submitting node an average of 5 weeks after case acceptance onto the system. Cases receive 3-12 expert reviews, with diagnoses and investigations being suggested for 98% and 88% of cases, respectively.

The DDS suggested diagnosis has been confirmed at molecular level for one case and there have been 2 separate suggestions of new recessive conditions based on affected sibling pairs. Five cases have also had a consensus confirmed clinical diagnoses. Overall the diagnostic rate for DDS cases is 15%.

These results show that the DDS is a workable and effective diagnostic system facilitating timely and equitable access for clinicians from across Europe to expert opinions.

P02.073 Application of western blot for analyzing of dystrophin in Iranian patients with mild dystrophinopathy

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Introduction: Dystrophinopathies (Duchenne muscular dystrophy and Becker muscular dystrophy) are X-linked recessive disorders manifesting with muscle degeneration and weakness. The gene which is defective in dystrophinopathies is the largest known gene, consisting of almost 0.1% of the human genome (2.500 kbp). The product of this gene in normal muscle, dystrophin, is a 427 kDa rod-shaped protein. The value of analyzing dystrophin on western blots of skeletal muscle for the differential diagnosis of Xp21 muscular dystrophies is now well established especially for mild forms of the disease (BMD) which immunohistochemistry techniques are not sufficient for the definite diagnosis.

Material & Methods: Here we describe a sensitive system based on monoclonal antibodies against dystrophin. System has been set up using GAPDH protein as control that extracted from k562 cells. In our study which was the first application of western blot analysis in muscle disorders in Iran, we examined muscle sample taken from 10 clinically suspected BMD first by immunohistochemistry methods and then by western blot analysis.

Results: Clinically and immunohistochemically suspected cases of BMD were confirmed by western blot analysis. These findings are not evident in other forms of muscular dystrophies (Limb-Girdle MD) which are closely resemble to BMD clinically.

Conclusion: Results show the necessity of blotting techniques in diagnosis panel of mild forms of dystrophinopathies.

P02.074 Ebstein anomaly: genetic heterogeneity and association with microdeletions 1p36 and 8p23.1

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Ebstein anomaly (EA) is an uncommon congenital heart defect (CHD), characterized by downward displacement of the tricuspid valve into the right ventricle. Aim of the study was: 1) search for chromosomal imbalances (using standard cytogenetic and array-CGH analysis) and single gene conditions associated with syndromic EA; 2) screening of *GATA4* and *NKX2.5* mutations in patients with non-syndromic EA. Between January 2000 and October 2009, 44 patients with EA were enrolled. Syndromic EA was found in 12 (27%) patients, non-syndromic EA in 32 (73%). A distinct syndrome was diagnosed by clinical criteria in 7 individuals (CHARGE syndrome in 2, VACTERL association, Noonan, Kabuki, Holt-Oram, and Cornelia de Lange syndromes each in one patient). In one syndromic patient a 18q deletion was diagnosed by standard cytogenetic analysis. Array-CGH analysis in 5 syndromic patients disclosed an interstitial deletion at 8p23.1 in one subject, and a del 1pter>1p36.32 /dup Xpter->Xp22.32 in another case. No mutation in *GATA4* and *NKX2.5* genes were detected in 11 non-syndromic patients studied. In conclusion: 1) EA is genetically heterogeneous; 2) the present study and published data suggest the following: a) del 1p36 and del 8p23.1 are the more frequent chromosomal imbalances associated with EA; b) likely candidate genes include *GATA4* (patients with del 8p23.1), *NKX2.5* (published patients with isolated EA), and an hypothetical locus in the 1p36 region (patients with del 1p36).

P02.075 Cloacal impaired development associated with multiple malformations in a young girl - case report

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Background: The ectopic anus is among the five most important types of cloacal malformations. Its incidence is higher in females. Occasionally, several anomalies exist, producing complex malformations. Some patients present associated congenital defects of mesonephric origin. The direct cause of the majority of these anomalies is unknown. Viral infections, teratogenic drugs, metabolic alterations and placental anomalies could be involved. The karyotype is generally normal. A superficial approach of these patients can facilitate the misdiagnosis of the associated defects. Material and methods: We present a girl aged 4 years, diagnosed with anal ectopia since neonatal period. Pediatric and surgical follow-up was done for a three years period. In our department, by persevering assessment, the case investigation was fulfilled. Anamnesis, clinical examination, genetic evaluation, biological and imaging studies were done. Results: Abdominal ultrasonographic examination identified agenesis of the right kidney. Cardiac echography detected atrial septal defect. Abdominal and pelvic MRI specified congenital single left kidney, normal urinary bladder and uterus, inguinal right ovarian ectopia. Standard karyotype couldn't exclude some anomalies under optical resolution limit. Conclusions: Associated malformations research was initiated due to the presence of the anal ectopia. Multifactorial mechanism could create propitious circumstances for these anomalies. Patient's interdisciplinary integrated management is essential. Further genetic evaluations and counselling are required.

P02.076 Congenital heart defects in Ellis van Creveld syndrome

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Ellis-van Creveld syndrome (EVCS) is an autosomal recessive skeletal dysplasia characterized by short ribs, short limbs, postaxial polydactyly, dysplastic nails and teeth, frequently associated with congenital heart defects. We present four cases in order to illustrate this rare entity and to discuss the variability of heart defects.

H.B.I. (5,8 y old female): disproportionate dwarfism, dysmorphic craniofacies (sparse, fine hair; prominent metopic suture, accessory gingival frenulae, conical teeth), narrow thorax, pectus carinatum, distal limb shortening symmetrically affecting the forearms and lower legs, brachydactyly and bilateral postaxial polydactyly, hypoplastic nails, fused proximal and medium falanges (finger II - V); ecocardiography: ASD ostium secundum, pulmonary valvular stenosis, hypertrophic cardiomyopathy.

T.L.A. (1,6 old male): disproportionate dwarfism, craniofacial dysmorphia (sparse hair, accessory gingival frenulae), narrow thorax, distal

limb shortening symmetrically affecting the forearms and lower legs, brachydactyly and bilateral postaxial polydactyly, hypoplastic bilateral fibulae; ecocardiography: large ASD ostium primum, cleft anterior mitral valve, hypoplastic tricuspid valve.

P.G.M. (6 days - deceased): disproportionate dwarfism, dysmorphic craniofacies (accessory gingival frenulae, neonatal teeth), narrow thorax, distal limb shortening symmetrically affecting the forearms and lower legs, bilateral postaxial polydactyly (feet); echocardiography: ASD, enlarged right ventricle.

T.M. (5y old, female): disproportionate dwarfism, craniofacial dysmorphia (accessory gingival frenulae, conical teeth), distal limb shortening symmetrically affecting the forearms and lower legs, brachydactyly and bilateral postaxial polydactyly, hypoplastic nails.

In conclusion, we present four cases of EVCS in order to illustrate this rare genetic disorder but also to discuss the variable expression of heart defects, long term follow-up, management and genetic counseling.

P02.077 Study of a lot of newborn with extremely low birth weight and malformative association

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Extremely low birth weight is defined as a birth weight less than 1000 grams. Extreme prematurity implies high risk of perinatal mortality and neonatal morbidity.

Material and method: The study was carried out in the Prematurity and Neonatology Department during two years, on a group of 20 premature newborns with birth weight under 1000 grams (800 grams-1000 grams), with gestational age between 27-32 weeks and follows the congenital malformation association at this lot.

Results: In the group the distribution by sex show a number of 11 (55%) male newborns and 9 (45%) female newborns.

The patent ductus arteriosus was revealed by ultrasound in 4 cases (20%), in 2 cases was associated with septal atrial defect. In 1 case septal atrial defect was associated with septal ventricular defect. In 2 cases septal atrial defect had no association.

At 1 case was present agenesis of corpus callosum diagnosed by transfontanelar ultrasound.. One single case presented plurimalformative syndrome with cheilognatopalatoschisis, varus equin and congenital heart disease but he died in the 3rd day of life.

Conclusions: Extreme prematurity is an important risk factor in increasing neonatal morbidity and mortality and requires care in intensive care unit; association of congenital malformations increases that risk and influences further development.

P02.078 Genetic heterogeneity in the Facio-Audio-Symphalangism syndrome

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In the past years it has become clear that many aspects of development are regulated by opposing activities of secreted ligands and their intra-and extracellular antagonists. This is also the case in the BMP signaling in vertebrates. The BMPs (Bone-Morphogenetic Proteins) are multifunctional proteins, and they are very important in bone and cartilage formation. Mutations in several genes involved in this cascade have shown to be responsible for many syndromes with synostosis/ symphalangism.

One of these genes causing symphalangism is the NOG gene on chromosome 17q21-22, coding for the polypeptide Noggin, which binds and antagonizes BMP 2, 4 and 7.

Noggin also interacts with GDF-5 (Growth differentiation factor 5). Recently mutations have also been described in the GDF-5 gene in families with symphalangism, and also in the BMP receptor BMPR1A to which GDF-5 binds. This binding leads to activation and regulation of transcription of target genes involved in bone formation.

Very recently also a mutation in another growth factor (FGF9) was found in a family with multiple synostosis.

We report a patient with a clinical phenotype that is very suggestive of the Facio-Audio-Symphalangism syndrome. Patients with this syndrome show proximal symphalangism, in combination with deafness due to stapes ankylosis, and a typical face, with a broad, hemicylin-

drical nose. We could however not show a causative mutation in the genes that are thusfar known to cause this phenotype, giving evidence for further genetic heterogeneity of this syndrome.

P02.079 Involvement of the neuromuscular junction in fetal akinesia deformation sequence (FADS)

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Introduction: Recently, neuromuscular junction (NMJ) genes have been implicated in FADS, mainly in multiple pterygium syndromes, lethal (LMPS) or not (Escobar syndrome). Recessive mutated genotypes have been identified in *CHRNG*, *RAPSN*, *CHRND*, *CHRNA1* and *DOK7*.

Patients and Methods: We received 32 patients with FADS for analysis of NMJ genes. Among those patients, 8 were alive and had arthrogryposis (+/- pterygiums), 21 foetuses had FADS leading to pregnancy termination, 1 foetus died *in utero*, and 5 patients died in the first year.

An analysis of *CHRNG*, *RAPSN*, *CHRND*, *CHRNA1*, *DOK7* and *MUSK* genes was undertaken by PCR-sequencing, in order to (i) determine etiological diagnosis (ii), estimate the frequency of NMJ dysfunction and (iii) try to determine genotype-phenotype correlations.

Preliminary results: Three patients were identified with biallelic mutations of *CHRNG*, coding for the foetal sub-unit of the acetylcholine receptor. Two of these patients were children diagnosed with Escobar syndrome and one was a foetus with LMPS (pregnancy termination at 13 weeks). Different types of mutations were identified: stop, splice or frameshift, either at homozygous or compound heterozygous state. A heterozygous frameshift mutation of *DOK7* was identified in a foetus with FADS and pterygiums (pregnancy termination at 22 weeks). Studies are underway to find a second mutation.

Conclusion-Perspectives Screening of NMJ genes is still underway in our cohort. Genetic confirmation is important for genetic counselling. However, up to date, sequencing of the NMJ genes has a low yield of positive diagnoses. Therefore, its use in FADS diagnostic algorithm remains to be defined.

P02.080 A Fragile X family with mitotically unstable premutation/full mutation mosaics.

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Fragile X syndrome (FXS) is caused by CGG triplet expansion in the first exon of *FMR1* gene >200 repeats. In childhood, full mutation associates with mental retardation, characteristic behavior and physical phenotype. Premutation (55-200 CGG) carriers have a higher risk of developing premature ovarian failure and/or fragile X associated tremor ataxia syndrome. About 15-40% FXS patients show mosaic pattern of full mutation and premutation.

Here we report a FXS family with mitotically very unstable expansion in both premutation and full mutation region in three generations. Molec-

ular analysis was performed with Fragile X PCR Test kit (Abbott) and with methylation-sensitive Southern blot using the StB12.3 probe.

Two children with FXS in this family showed mosaic pattern of premutation and full mutations. The boy presenting mental retardation and behavioral problems at age 7 had expanded fragments with 81-370 CGG repeats. The girl showed 135-950 CGG repeats in the expanded allele of the *FMR1* gene and had only 5-10% normal allele band intensity. Their mother showed full mutation between 350-460 CGG repeats and had normal allele activated in 95% of blood cells. Mitotically unstable expansion around 600 repeats and normal allele activation about 90% was detected on her sister. The grandmother had mosaic pattern of premutation of 102, 109 and 186 CGG repeats and clinically presents signs of mood lability and anxiety.

Molecular investigation of mitotically unstable expansions is challenging and requires combining of PCR with Southern hybridization analyses. The latter allows to discover large mosaic fragments and to study methylation patterns

P02.081 New *ALX4* phenotype due to novel homozygous mutation: p.Q225E

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Frontonasal Dysplasia (FND;OMIM#136760) results from abnormal frontonasal development. Craniofacial anomalies in FND include anterior cranium bifidum, hypertelorism, orofacial clefting, notching of alae nasi. Most cases are sporadic and *EFNB1* is the first gene identified to be responsible from the X-linked subgroup, Craniofrontonasal Dysplasia (CFND;OMIM#304110). Autosomal dominant mutations in *MSX2* and *ALX4* have been associated with parietal foramina. Recently, autosomal recessive (AR) mutations in *ALX3* were associated with frontorhiny, while AR *ALX4* cases were shown to have additional features of alopecia, genital anomalies, craniostenosis.

Here we report a 6,5 year-old boy, who presented with distinctive facial appearance; severe hypertelorism, wide-bifid nasal tip, anteverted nares, broad columella, notched alae nasi, unilateral preauricular tag, broad frenulum, missing permanent left upper incisor, bilateral parietal foramina of 3x3 cm. He was born to consanguineous parents after an uneventful pregnancy and delivery. His motor milestones were achieved within normal limits and he has good school performance. Physical examination prompted the clinical diagnosis of frontorhiny associated with parietal foramina.

EFNB1, *ALX3*, *ALX4*, *MSX2* genes were sequenced and homozygous p.Q225E mutation, changing neutral-polar glutamine to acidic-polar glutamic acid in the homeobox domain, was found in *ALX4*. This variation was not identified in 100 control chromosomes.

To our knowledge, this is the second recessive mutation identified in *ALX4* gene. The first reported recessive *ALX4* mutation (p.R265X) was responsible from an atypical frontonasal malformation in a large consanguineous Turkish family. Our findings demonstrate that *ALX4* cases may show variable clinical expressivity with overlapping features of *ALX3* and *MSX2* phenotypes.

P02.082** Geleophysic dysplasia is a clinically and genetically heterogeneous disease

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Geleophysic dysplasia (OMIM 231050) is an autosomal recessive disorder characterized by short-limb dwarfism, brachydactyly, and a 'happy-looking' facial appearance. It is frequently associated with cardiac valvular disease although the incidence and natural history of the heart complications remain unclear. Mutations in the *ADAMTSL2* gene

resulting in dysregulation of TGF- β signaling have been recently recognized to be responsible for this condition. We screened for *ADAMTSL2* mutations fourteen cases of geleophysic dysplasia diagnosed on the basis of clinical and radiological findings. Novel pathogenic mutations were found in three of the fourteen cases suggesting that additional and yet unknown gene(s) are involved in the pathogenesis of the disease. Short stature and brachydactyly were present in most of the cases while laryngeal stenosis, cardiac disease, and Perthes disease were variably present. Taken together, these findings show a significant clinical variability and are consistent with a broad disease spectrum. In 3 of 4 cases in which skin fibroblasts were available, we detected by electron microscopy cytoplasmic lysosome-like inclusions which have been previously reported as a characteristic finding of the disease. Inclusions were also detectable in lymphoblasts in one patient with mutations in *ADAMTSL2*. Analysis for glycosphingolipids in geleophysic dysplasia fibroblasts did not reveal significant abnormalities compared to control cells. Therefore, the nature of the accumulated material remains unclear and whether TGF- β signaling plays a role in the pathogenesis of the inclusions requires further investigations.

P02.083 *GJB2* (Cx26) mutations and hearing impairment in Southern Finland - experiences from a diagnostic genetic laboratory

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Hearing impairment is the most common sensory disorder and it is present in approximately 1 of every 500 newborns. The most frequent gene causing autosomal recessive nonsyndromic hearing loss (ARNSHL) is *GJB2* (Cx26) and its mutations are responsible for 30-60% of congenital ARNSHL. There is a single common mutation, c.35delG in *GJB2* that may account for up to 70% of all *GJB2* mutations.

Our diagnostic laboratory has offered *GJB2* mutation screening since 2004 as either targeted screening for the most common c.35delG mutation or full-sequencing analysis of *GJB2*. Using the patient samples with diagnosis of hearing impairment, referred for us for *GJB2* mutation screening during the years 2004-2009, we wanted to investigate how commonly mutations were identified, what was the mutation spectrum and if there were any obvious genotype-phenotype correlations. Majority of the patients were Finnish.

Results: Altogether 125 patient samples had been analysed in our laboratory and *GJB2* gene mutations were identified in 33 patients (26%, n=33/125) confirming diagnosis of ARNSHL. Of the mutation positive patients, 22 (67%, n=22/33) were homozygous for the c.35delG mutation, 10 (30%, n=10/33) were compound heterozygous and 1 patient was homozygous for the c.71G>A (p.Trp24X) mutation. The following six mutation combinations were identified in compound heterozygous patients: c.35delG + c.101T>C (p.Met34Thr), c.35delG + c.269T>C (p.Leu90Pro), c.35delG + c.456C>G (p.Tyr152X), c.35delG + c.550C>T (p.Arg184Trp), c.427C>T (p.Arg143Trp) + c.101T>C (p.Met34Thr) and c.109G>A (p.Val137Ile) + c.233delC (p.Leu79CysfsX3). The patients with compound heterozygosity seemed to have either later onset or diagnosis of hearing impairment.

P02.084 High correlation of MRI, Chemical and Genetic finding of Glutaric Acidemia type 1

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Background and aim: Glutaric acidemia type 1 (GA-1), one of the most common organic acidemia, and autosomal inheritance, is due to deficiency of glutaril-CoA dehydrogenase. Patients are often macrocephalic and develop other signs before age of 48 months. MRI shows widening of Sylvian fissure, acute striatal degeneration and shrinkage of caudate and putamen. High concentration of glutaric acid in the urine, body fluids is usual. Treatment before symptoms presentation may prevent brain damage in most patients.

Material and method: Five cases in four families were studied, three girls with ages of 24, 27 and 31 months with clinical symptoms. The family with history of two deceased children was the last.

Results: In two cases no definite diagnosis was achieved in neurology surveys. But MRI report had exactly proved GA-1 as the most probable

diagnosis. Enzyme study suggested the same, and finally *GCDH* gene molecular analysis revealed a missense mutation at codon 181. In other case, whose sister was deceased with just the same symptoms at, MRI study suggested (GA-1) as the first diagnosis, and then organic acids in urine showed the typical pattern for the disease. In third case, the MRI diagnosis and enzyme study had suggested (GA-1) the same. In the girl with 27 months age compound heterozygosity for *GCDH* gene at codon 181 & 255 was observed. In two disease cases with high GA we found no mutations in *GCDH* gene.

Conclusion: The results suggest that exact study of MRI in suspicious patients could help in diagnosis.

P02.085 Goldenhar syndrome-possible microdeletion syndrome?

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Goldenhar syndrome (oculoauriculovertebral dysplasia) is extremely variable congenital defect with incidence 1:3 000- 1:5 000. It results from the anomaly of the first and second branchial arches and is characterized by unilateral deformity of external ear and small ipsilateral half of the face, praearicular tags, epibulbar dermoid and vertebral anomalies. Most cases are sporadic, some families clearly support autosomal dominant and rarely autosomal recessive inheritance. The exact cause is not known, but it is possible, that it is caused by microdeletion.

We present the collection of 6 patients with Goldenhar syndrome and 2 patients with microtia - atresia syndrome, which we intend investigate by Illumina SNP array.

P02.086 Co-occurrence of severe Goltz-Gorlin syndrome and pentalogy of Cantrell - clinical and molecular analysis.

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Goltz-Gorlin syndrome is a highly variable multisystem defects of meso-ectodermal origin. Major manifestations include atrophic skin lesions with fat herniation, papillomas, ocular defects, hypodontia, hearing loss, limb malformations. Mutations in the X-linked PORCN gene can be identified in all patients with a classical Goltz-Gorlin phenotype.

The pentalogy of Cantrell is a specific combination of usually five anomalies: a midline abdominal wall defect; defect of lower sternum; deficiency of the diaphragmatic pericardium and anterior diaphragm and congenital heart anomalies. Cantrell pentalogy is infrequently reported. The pathogenesis is still unknown.

We report an infant with both findings fitting Cantrell pentalogy (lack of the lower sternum; diaphragmatic hernia; ectopia cordis; omphalocele) and other findings fitting Goltz-Gorlin syndrome (sparse hair; anophthalmia; clefting; bifid nose; irregular vermillion of both lips; asymmetrical limb malformations; caudal appendage; linear aplastic skin defects; unilateral hearing loss). She shows a psychomotor and somatic retardation.

Genotype analysis showed a normal karyotype, absence of subtelomeric deletions and CGH imbalances, but presence of a PORCN mutation, which confirmed the diagnosis of Goltz-Gorlin syndrome.

This combination of Goltz-Gorlin syndrome and pentalogy of Cantrell has been reported recently in two patients with a confirmed PORCN mutation. The present patient confirms that the pentalogy of Cantrell is caused at least in some cases by a PORCN mutation. It remains as yet uncertain whether this can be explained by the type of localization of the mutation within PORCN, or whether the phenotype is additionally determined by mutations of polymorphisms in other genes, environmental factors, and/or epigenetic influences.

P02.087 Gorlin syndrome - case report

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Introduction: The nevoid basal cell carcinoma syndrome or Gorlin-Goltz syndrome is an autosomal dominant inherited disease with complete penetrance, intra and interfamilial variation in expression and no evidence of anticipation. The syndrome is characterized by the development of multiple, recurrent jaw odontogenic keratocysts (90%), frequently beginning in the second decade of life, multiple basal cell carcinomas, frequently on the face, the back, and the chest, hyperkeratosis of palms and soles, ectopic calcifications (particularly intracranial), skeletal abnormalities, facial dysmorphism, ocular disorders, cardiac and ovarian fibromas. The diagnosis is established using clinical diagnostic criteria. PTCH, located on chromosome 9q22.3-q31, is the only gene known to be associated with NBCCS and normally functions as a tumor suppressor gene, controlling growth and development of the normal tissues. Molecular genetic testing, available on a clinical basis, detects mutations in the majority of affected individuals.

Case presentation: A 30 years old female presented with unilateral enlargement of the mandible and no other clinical findings in physical examination.

Radiological examination revealed unilateral multicystic lesions of the lower jaw. Histological examination demonstrated the presence of multiple unilateral odontogenic keratocysts

The patient presented in the past other three similar lesions of the lower jaw and two facial basal cell carcinomas.

Family history identified other two members with similar lesions.

Conclusion: The case present two major diagnostic criteria for Gorlin syndrome.

The patient will require, long-term follow-up, treatment of the new or recurrent manifestations and molecular genetic testing to detect PTCH mutation.

P02.088** Cephalopolysyndactyly syndrome with agenesis of the corpus callosum: The contribution of array comparative genomic hybridisation

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In 1928, Greig reported a rare multiple congenital anomaly syndrome named Greig cephalopolysyndactyly (GCPs - MIM # 175700) characterised by hypertelorism, macrocephaly with frontal bossing, poly-syndactyly in the upper and lower limbs and other findings including central nervous system malformations with variable cognitive impairment. The pattern of inheritance is autosomal dominant by loss of function mutations in the *GLI3* (7p14) transcription factor gene. Later, other cephalopolysyndactyly clinical variants have been reported (e.g. the acrocallosal syndrome (ACS) (MIM #200990), ...) expanding the clinical spectrum and making the differential diagnosis challenging since the findings in GCPs are relatively non-specific. The ACS has substantial overlap with GCPs including mental retardation, preaxial polysyndactyly, facial dysmorphism with macrocephaly, prominent broad forehead and hypertelorism, agenesis of the corpus callosum and mental retardation. It is autosomal recessive but many cases are sporadic. One patient had a mutation in *GLI3*.

Here we report a series of 15 patients presenting with a cephalopolysyndactyly and agenesis of the corpus callosum. Conventional karyotyping, FISH using the BAC clone RP11-706L12 (7p14) and sequencing *GLI3* were all normal. Subsequently, we performed array comparative genomic hybridisation (aCGH) using a 1 Mb resolution BAC aCGH and a 200 kb resolution oligo aCGH (105k OGT). We identified causal genomic rearrangements in 3 patients (i.e. a complex chromosomal rearrangement involving chromosomes 1 and 21q, a dup(X)(q13.3;q21.1), and a deletion on chromosome 22q13.1). Clinical and genotyping correlations will be discussed together with the value of aCGH in the molecular diagnosis workup of CPS of unknown aetiology.

P02.089 MODY type 2 with Greig Cephalopolysyndactyly Syndrome (GCPs) patient - a part of a contiguous gene deletion syndrome (GCPs-CGS).

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The Greig Cephalopolysyndactyly Syndrome (GCPs) is a rare (1-9/1,000,000), pleiotropic, multiple congenital anomaly syndrome that is inherited in an autosomal dominant pattern. The primary findings include a triad of digital anomalies, ocular hypertelorism, and macrocephaly. Other low frequency findings include central nervous system (CNS) anomalies, hernias, and cognitive impairment. GCPs is caused by loss of function mutations in the *GLI3* transcription factor gene, located on chromosome 7p13. These include point mutations, frameshift mutations, translocation, and gross deletion mutations. Treatment include plastic or orthopedic surgery of limb malformations when needed and care by child developmental team if there is cognitive impairment. Contiguous gene syndrome (CGS) have been previously reported to be associated with GCPs often with additional problems related to adjacent genes on chromosome 7p13. Some CGS have stereotypical breakpoints while others not. In most CGS, the extent of the deletions is correlated with the severity of the symptoms. To date, several reports of GCPs-CGS have been published with no common/hotspots breakpoint.

We present a 9 y old girl who presented with clinical diagnosis of GCPs with mental retardation and newly onset diabetes mellitus. Laboratory work-up was compatible with a diagnosis of maturity onset diabetes of the young (MODY). A contiguous gene deletion syndrome was suspected. Chromosomal analysis revealed a 46,XX,del(7)(p13;p15) karyotype. The deleted area includes the glucokinase gene (GCK) causing MODY type 2 located at 7p15-p13.

This is the second report of MODY in GCPs-CGS patient.

Our results further emphasize the importance of chromosomal analysis in cases of GCPs.

P02.090 Role of connexin genes (Cx26, Cx30 and Cx31) in hereditary hearing loss

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Connexins are the key components of the gap junction, which regulates various physiological and development processes. The importance of the gap junction for human physiology has been identified as a result of detection of multiple genetic diseases that are associated with different connexin genes. Hearing loss is the most important in terms of incidence. Since mapping and identification of Cx26 gene we conducted comprehensive medical- and population-genetic studies in several regions of Russia. According to recent data, mutations in different connexin genes are associated with non-syndromic hereditary hearing loss (NSHL). In some populations of Europe and Asia mutations in these genes are described in 70-90% cases of all NSHL patients. DNA studies of NSHL patients in Russia were made only for the Cx26, while identification of mutations in other connexin genes has not been previously conducted.

This study aims to determine the spectrum of mutations in Cx26 and Cx31 and two big deletions - del(GJB6-D13S1830) and del(GJB6-d13S1854) in Cx30. 240 NSHL patients from Kirov region were analyzed. Different mutations in Cx26 gene were found, and 35delG (25,2%) was the most frequent. 150 patients with no mutations or heterozygous in the Cx26, were analyzed at Cx31. Mutations in Cx31 798c>t (13,67%), R32W (4,67%), and 357c>t (3,67%) were present in a large number of cases. Deletions in Cx30 were not found in our group of patients.

We plan to conduct further analysis of connexin genes and create a system for searching mutations for NSHL patients based on phenotypic manifestations.

P02.091 Application of Chromosomal Microarray Analysis in Patients with Early Onset Hearing Loss and Mild Dysmorphism

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The aim of our study was to find out new etiological causes for early onset hearing loss (HL). We performed whole-genome analysis in 24 children with still unknown HL and mild dysmorphism and/or other developmental complaints.

Our study group consisted of 233 children with early onset HL as a main complaint collected during 2000-2009. Of the 233 investigated probands we found 126 patients (54%) with *GJB2* or other gene mutation(s). The clinical data of the rest of this investigation group was carefully reexamined. We selected out 24 patients who had in addition to early onset HL subtle facial dysmorphism, failure to thrive and/or developmental or behavioral problems. In order to detect copy number changes in selected patients, whole-genome genotyping was performed, using the Illumina platform. Three regions in three separate patients with the loss of one allele were found. All findings were confirmed by quantitative PCR analysis.

One 8.5-year-old girl has peculiar facial phenotype, developmental delay, mild sensorineural HL (SNHL) and ~2.94-Mb size deletion in chromosomal region 12q13.3-q41.1. She has haploinsufficiency of cochlear-expressed *MYO1A* gene (DFNA48). The 10-year-old girl with subtle facial dysmorphism and mild SNHL has ~0.74-Mb size deletion in 3p26.2 region, which she inherited from her father. In this region is located *DFNB6* locus, however, the association between patient's phenotype and deletion is unclear, as her father has normal hearing. Third patient (10-year-old) has severe SNHL, subtle facial dysmorphism and 0.54-Mb size deletion in 1p33 region. There are not known previously identified SNHL locus in this area.

P02.092 A Report of Three Egyptian Patients with Hereditary Osteolysis

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Introduction: Diseases exhibiting osteolysis in children are rare hereditary disorders. Osteolysis disorders are characterized by destruction and resorption of affected bones. Several types have been recognized with different clinical manifestations. Subjects & Methods: The current study added three new cases from two unrelated consanguineous families with severe form of inherited osteolysis. Meticulous history taking, clinical examination, radiological and biochemical investigations are included. Results & Conclusions: The clinical and radiological findings in our patients were best compatible with the diagnosis of Winchester syndrome (WS) and Torg/ Nodulosis-arthropathy-osteolysis syndrome (Torg/NAO). Our findings confirm that Torg/NAO and WS represent a continuous clinical spectrum. The present study is a great opportunity for further molecular analysis to emphasize that Torg/NAO and WS are allelic disorders causing the clinical and radiological overlapping manifestations.

P02.093 Complete screening of *SPG4* and *SPG3A* in a Spanish patient cohort.

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Hereditary spastic paraparesis (HSP) comprises a group of inherited neurodegenerative disorders with weakness and spasticity of the limbs (pure) or additional features (complicated). HSP shows genetic heterogeneity and can be autosomal-dominant (AD-HSP), autosomal-recessive and X-linked-recessive. At least 45 different genes/loci can cause HSP. Mutations in *SPG4* and *SPG3A* account for approximately 40% and 10% of all AD-HSP cases, respectively. The frequency of mutations in the Spanish population needs to be explored. Our preliminary study of 43 families indicated a high frequency of *SPG4*. Now we present the results of the complete analysis of an extended series of 92 Spanish patients (67 families: 46 from Galicia, 21 from other regions of Spain). We performed direct sequencing of the whole coding region of *SPG4* and *SPG3A*. Additionally, we searched for small and medium size structural abnormalities in both genes by MLPA. Ten mutations

were detected in *SPG4*, six of them new. We identified two previously described mutations in *SPG3A* in two families with pure AD-HSP and childhood onset. All new sequence variations were screened for in 250 neurologically normal control individuals. We confirm the high prevalence of *SPG4* among AD-HSP also in Spain (21% of all cases, 52% of the AD-HSP). The frequency of mutations in *SPG3A* was similar to previous reports (3% of all cases, 7.4% of AD-HSP). Together, screening of *SPG4* and *SPG3A* provides a diagnostic confirmation in over 25% of all HSP and over 60% AD-HSP families and should be the first step in the study of these patients.

P02.094 Craniofacial syndromes caused by endoplasmic reticulum export defects

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We identified a novel autosomal recessive syndrome, cranio-lenticulo-sutural dysplasia (CLSD, Boyadjiev-Jabs syndrome) due to SEC23A missense mutations. Characteristic features of this disease are late-closing fontanels, sutural cataracts, facial dysmorphisms and skeletal defects. Prominent cellular features of skin fibroblasts from these patients are secretion defect and marked distension of the endoplasmic reticulum (ER), concordant with the function of SEC23A in protein export from the ER. Knockout mice model for a partner of SEC23A within the COPII mediated vesicular export of secretory proteins from the endoplasmic reticulum exhibit an embryonically lethal phenotype of holoprosencephaly and severe craniofacial anomalies with the spectrum of Pierre Robin-otocephaly phenotype. Our results suggest that deficiency of COPII components cause craniofacial and brain phenotypes that have thus far escaped classification.

P02.095 Hereditary spastic paraparesia in 25 Egyptian families

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Hereditary spastic paraparesis (HSP) is characterized by progressive spasticity of the lower limbs that can be inherited in an autosomal recessive, autosomal dominant or X-linked manner. The heterogeneity of the disease is not only demonstrated by the various forms of HSP, but also by the numerous HSP loci that have already been identified. We have recruited 63 patients from 25 Egyptian families with complicated or uncomplicated forms of recessive HSP to identify additional HSP loci. One of these families with apparent X-linked inheritance displayed a mutation in *PLP1* gene. We excluded linkage to known loci in 15 families, which were subsequently evaluated by genome-wide 5K SNP linkage scan to identify new loci. Positive consanguinity was in 23 families (92%) and patients' age ranged from 2 years to 40 years. The onset varied from 1 year to 18 years and complicated HSP was in 17 families. The only presenting symptom in uncomplicated HSP was spasticity of lower limbs while in complicated HSP there were variable manifestations in the form of dysarthria, mental retardation, nystagmus, peripheral neuropathy and acropathy with self mutilation that was excluded from linkage to *Cct5* gene. Neuroimaging findings were thin corpus callosum, defective myelination, cerebellar atrophy and retrocerebellar cyst. Our analysis revealed that 8 families defined novel HSP loci, and 2 families mapped to known loci. These results demonstrate the genetic heterogeneity of the disease and that there are still many more causes yet to be explored. Our cohort shows the likelihood of identifying more novel HSP genes.

P02.096 Molecular evidence of chimera formation resulting from parthenogenetic division and dispermic fertilization

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Whole-body human chimerism is the result of two fertilization events giving rise to one individual, and is a rarely detected condition reported in the literature. We have studied the molecular background and discuss the likely mechanism for the detected chimerism in a patient with a 46,XX/47,XY,+14 karyotype and ambiguous genitalia, cryptorchidism, pigmentary anomalies and normal psychomotor development. We have used karyotyping, interphase-FISH and array CGH analysis as well as molecular analysis of polymorphic markers at 48 loci in order to define the origin and percentage of 47,XY,+14 cells in different tissues. Our results indicate that the chimerism in our patient is a result of dispermic fertilization of a parthenogenetically activated oocyte. Our report underlines that cytogenetic findings suggesting mosaicism might actually indicate chimerism as an underlying cause in patients. It also highlights the difficulties in predicting the clinical outcome in patients with genetic aberrations in mosaic or chimeric form.

P02.097 An emerging chromosome X duplication hot-spot, Xp11.21, involved in developmental delay; two new families

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To date, six unrelated families presenting with predominantly nonsyndromic developmental delay have been reported with a microduplication of chromosome Xp11.2 (Am J Hum Genet. 2008;82(2):432-43). Here, we present two additional families with similar/overlapping Xp11.2 duplications. The first family consists of two boys, aged 8 and 10, who presented with triangular face, prominent forehead, high palate and developmental delay. Microarray-comparative genomic hybridization (array-CGH) identified a 513 kb duplication at Xp11.22 in this family. The second family consists of a boy, age 7, with mild facial features and developmental delay. G-band analysis showed an apparent duplication of band Xq21.2 that was maternally inherited. Array-CGH and FISH revealed a 4.6 Mb duplication of the Xp11.21-p11.22 region that inserted into the X chromosome at Xq21.2. Comparison of the duplicated region in the eight families revealed that the minimal, commonly duplicated region encompasses the *HUWE1* gene. *HUWE1* encodes an E3-ubiquitin ligase involved in neuronal cell cycle and previously linked to developmental delay. Other commonly duplicated genes in this region linked to X-linked developmental delay include *PHF8*, *HSD17B10*, and *JARID1C*. Our study underscores the utility of metaphase FISH to acquire positional information of the genomic imbalances revealed by array-CGH and provides support that increased gene dosage of *HUWE1* and *PHF8*, or both, contribute to the etiology of this disease. Finally, it is known that several genes in this region escape X-inactivation. We propose that the sequence elements that facilitate the escape of X-inactivation contribute to the genomic instability, namely, the recurrence of duplications, in this region.

P02.098 Haploinsufficiency of *IGF1R* is not always associated with short stature, ARVD can be caused by a deletion of the entire *PKP2* gene, and other things that I have learned from ordering arrays.

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Karyotype analysis was the standard of care for patients with developmental delays; however, arrays are now first line investigations in many clinical practices. More patients are receiving diagnoses as microdeletion/duplication syndromes are detected. With better coverage, smaller alterations are detected, which can help localize which genes may be responsible for a particular phenotype. Often these alterations contain only a single gene. From arrays that are ordered in a clinical practice, the results can add to our knowledge of clinical genetics. Examples of such insights include: arrhythmogenic right ventricular dysplasia (ARVD) can be due to a 112-kp deletion that contains the entire *PKP2* gene, a 1.3 Mb deletion at 15q26.3 containing *IGF1R* is NOT associated with short stature, there appears to be a facial phenotype associated with partial deletion of *NRXN1*, and the threshold for ordering arrays should be very low.

P02.099 Analysis of four patients withinv dup del(8): clinical, cytogenetic and molecular characterization of this alteration.

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Inverted duplication 8p associated with deletion of the short arms of chromosome 8 (inv dup del(8p)) is a complex chromosomal rearrangement relatively common, with an estimated prevalence in the general population of 1/10000-30000 liveborns. Clinical manifestations of this alteration include generally severe to moderate mental delay and typical facial features. Furthermore, there are associated malformations of the CNS such as hypoplasia/agenesia of the corpus callosum (80%), skeletal abnormalities (scoliosis/kyphosis) (60%) and congenital heart defects (25%) (Guo WJ. Am J Med Genet 58(3):230-6).

The rearrangement of the chromosome consists of a deletion of the telomeric region 8p23-pter, and an inverted duplication of the 8p11.2-p23 region. We present four children with this anomaly, which deletion and duplication size is variable and has been estimated by SNP array and array-CGH techniques. In the attached table, the deletion and duplication sizes and the highly variable clinical outcome of every one of the patients are correlated.

A microsatellite segregation study of the short arms of chromosome 8 in these patients has been made, detecting the maternal origin of this chromosome in all of them. The inversion of the duplication of the region within 8p11.2-p23 has been confirmed with FISH technique.

It is important to characterize the size of both, the deletion and the duplication in every patient, as this might be related with the severity of the clinical manifestations.

P02.100 Is it IPEX Syndrome? Case Report

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The authors present the case of a male patient aged 14 years old, diagnosed with polyendocrine autoimmune association (mellitus diabetes type 1, autoimmune thyroiditis and hypo-gonadotropic hypogonadism) by the occasion of keto-acidotic diabetes onset. This patient associated celiac disease, sustained on clinical, immunological and histological changes: recurrent diarrhea, positive IgA anti-endomysium and anti-tissue transglutaminase antibodies and total villous atrophy on intestinal biopsy sample. He also presented recurrent eczematous dermatitis associated to elevated serum concentration of immunoglobulin E. The authors sustain the diagnosis of IPEX syndrome in this case based on clinical and laboratory aspects presented, along with a positive family history of unexplained early deaths in the mother-side male relatives (two of patients' uncles). Immune Dysregulation, Polyendocrinopathy, Enteropathy X-Linked (IPEX) Syndrome represents a rare X linked disorder, characterised by development of systemic autoimmunity from the first year of life. Life expectancy is reduced, but there were described several cases as this one, associating late onset, that reached the second or the third decade of life. IPEX is a rare genetic autoimmune disease due to mutations in the *FOXP3* gene, located on the X-chromosome. There are no specific laboratory tests to confirm the diagnosis. Molecular analysis of the *FOXP3* gene (Xp11.2-q13.3) is required for the diagnosis. Drug treatment includes immunosuppression, nutritional support and hormone replacement. The unique definitive treatment for IPEX is hematopoietic stem cells transplantation.

P02.101 Unusual case of bilateral iridocorneal endothelial syndrome with extraocular anomalies

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Iridocorneal endothelial (ICE) syndrome is a rare chronic ocular disorder with three subtypes: Chandler's syndrome, Cogan-Reese syn-

drome, and essential (progressive) iris atrophy. It is characterized by abnormal corneal endothelium, iridocorneal adhesions, corectopia, iris atrophy and increased intraocular pressure. In up to 82% of cases it progresses to glaucoma. The ICE syndrome is usually unilateral, however some bilateral and a few familial cases have been reported. The etiology of this syndrome is unclear, but the most common theory thus far suggests a potential viral origin.

We report a new case of bilateral ICE syndrome in a 38-year-old woman with a family history (41 members) positive for genetic ocular diseases from the paternal side: pseudoexfoliation glaucoma (proband's uncle) and macular degeneration (proband's grandmother). Patient's main bilateral ocular symptoms were corectopia, iris atrophy and increased intraocular pressure. Gonioscopy of the right eye revealed also extensive iridocorneal adhesions and narrow, partly closed anterior chamber angle. Visual acuity and visual fields were normal. Unlike most reported ICE syndrome cases with an unremarkable medical history, our patient suffered also from endometriosis and allergic rhinitis, and had several extraocular facial anomalies such as prominent maxilla, retrognathia, triangular-shaped opened mouth and high palate, as well as skeletal anomalies like mild kyphoscoliosis. Karyotype 46,XX. In conclusion, both the previously reported bilateral and a few familial cases, as well as our patient with bilateral ICE syndrome and extraocular anomalies, indicate the possibility that genetic factors might be involved in the etiology of this syndrome.

P02.102 Jeune Syndrome: A Case Description Study.

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Jeune syndrome (OMIM 208500, Q77.2) is characterized by potentially lethal dwarfism, autosomal-recessive asphyxiating thoracic dystrophy with short horizontally ribs, short limbs, a respiratory distress which can be rapidly fatal. As well as is characteristic lung hypoplasia, polydactyly, cardiac anomalies, and renal failure. Incidence is 1 per 100000 - 130000.

A newly-born female child was born in 7. 07. 2009, admitted to hospital in 9. 07. 2009, and died in 23. 07. 2009. The newborn was from first 39-weeks pregnancy, bodyweight 3300, length 49 cm.

During the pregnancy in mother was diagnosed unilateral doubling of kidney, on the background of aggravation of chronic pyelonephritis.

The pregnancy was evidenced as late as in 22 weeks, and anomaly was diagnosed in fetus on 34 - 35 weeks of gestation, when was discovered skeletal malformation and was suspected hypochondroplasia.

On X-ray investigation from was diagnosed significant chest constriction with short ribs and secondary pulmonary hypoplasia, presumably Jeunes syndrome, cardiomegaly and signs of intrauterine pneumonia. the child was karyotyped with normal result.

On Doppler echocardiography - the presence of fetal communications, aortic coarctation and severe pulmonary hypertension.

On the 16-th day of life the status heavily worsened with progressive respiratory insufficiency and prominent bradycardia and following cardiac arrest.

In this case the prenatal diagnosis is possible if DNA diagnosis is available, and is recommended ultrasonographic scan of fetus during next pregnancy.

P02.103 Is hearing loss a feature of Joubert syndrome?

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Objective: to assess if hearing loss is a feature of Joubert syndrome (JBS), one of the ciliopathies and therefore possibly associated with hearing loss.

Study design: we retrospectively collected the audiological data of

Dutch JBS cases.

Results: data from 22 Dutch JBS cases (17 males, 5 females) aged 3 to 40 years were available. In 14 cases audiological investigations were successfully performed. Three cases (from 17 to 26 years old) showed very mild sensorineural hearing loss (SNHL) at different frequencies. Conductive hearing loss due to middle ear infections occurred frequently in young JBS children (6 out of 22 cases). In three cases (from 3 to 13 years old) the parents reported hypersensitivity to sound. In the five cases with two pathogenic *AHI1* mutations only one subject had very mild SNHL.

Conclusion: There is no evidence for significant hearing loss in Joubert syndrome. However, given the already compromised speech development in JBS, conductive hearing loss due to middle ear infections in young patients should be treated vigorously. SNHL at a later age cannot be excluded on the basis of our data, since three of the cases in the older age range showed discretely increased hearing thresholds. Analogous to the ciliopathy Bardet Biedl syndrome, where subclinically increased hearing thresholds were reported in a group of adolescents patients, follow-up of JBS patients is important in view of the possibility of progressive late onset SNHL.

P02.104 Bilateral mucinous cystadenoma of ovary in a patient with 10q23 microdeletion

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Juvenile polyposis syndrome (JPS) is a rare hereditary condition caused by mutations in SMAD4 or BMPR1A genes. Multiple juvenile polyps can also be found in a related group of syndromes with multisystemic involvement including Cowden disease, Lhermitte-Duclos disease, Bannayan-Riley-Ruvalcaba syndrome, and Proteus-like syndrome, all grouped as PTEN hamartoma tumor syndromes (PHTS). All forms of juvenile polyposis manifest in older childhood or early adulthood. Infantile juvenile polyposis is a rare entity, presenting in the first year of life with severe gastrointestinal symptoms. Many of these patients have associated macrocephaly, hypotonia, and congenital anomalies. It was recently recognized that patients with infantile polyposis have a 10q23 microdeletion, involving both BMPR1A and PTEN genes. There is a major risk for gastrointestinal malignancies in these patients, but the risk for development of other tumors is not known. We describe a patient with a history of infantile polyposis, macrocephaly, developmental delay, hypotonia and a 10q23 microdeletion. At age 14 she presented with bilateral mucinous cystadenoma of the ovary. This type of tumor was not previously reported in association with JPS, 10q23 microdeletion syndrome, or infantile polyposis. We believe that ovarian cystadenomas may be another neoplastic complication of infantile polyposis, and that our report widens the spectrum of the 10q23 microdeletion phenotype.

P02.105 Evidence for genetic heterogeneity of Keutel syndrome

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Keutel syndrome (KS) is a rare autosomal recessive disorder characterized by abnormal cartilage calcifications, peripheral pulmonary stenosis, midfacial hypoplasia, and brachytelephalangia. Mutations in the MGP gene have been identified in four families. X-linked chondrodysplasia punctata (CDPX1) is caused by ARSE deficiency, and has clinical overlap with KS. We present the clinical manifestations of five new patients from three families, with a clinical diagnosis of KS. Mutation analysis of the MGP and ARSE was performed. RESULTS: Three siblings born to a consanguineous Turkish couple had facial features and skeletal features of KS and carried a novel homozygous nonsense mutation, c.79G>T (E27X). A fourth male patient, of Korean ancestry had typical facial features, hearing loss and brachytelephalangia and a deletion of the entire ARSE gene was documented. The final female patient of South African descent had similar facial features, brachytelephalangia and laryngeal and tracheal calcifications, but no MGP or ARSE mutations were identified. CONCLUSION: MGP en-

codes vitamin K-dependent matrix Gla protein that acts as a calcification inhibitor by repressing BMP2. Despite the complete loss of MGP in the Turkish family, the mild clinical manifestations suggest functional redundancy of the mechanisms preventing cartilage calcification. Clinical overlap of KS and CDPX1 suggests that MGP and ARSE share a common biological pathway and/or substrate. As neither MGP nor ARSE mutations were identified in the South African patient, we suggest that further genetic heterogeneity exists for KS.

P02.106 Clinical heterogeneity in a large family with a mutation in *LMNA* gene

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Mutations in *LMNA* gene, which is located in 1q21.2 and codifies for twelve exons, can be considered to cause four different groups of overlapping disorders: 1) Diseases of striated muscle, 2) Lipodystrophy syndromes, 3) A peripheral neuropathy and 4) Accelerating aging disorders.

We present a large family including four generations, with limb girdle muscular dystrophy Type 1B LGMD1B showing a particular heterogeneity in the cardiological involvement. Eight patients present limb girdle muscular dystrophy, mainly in proximal lower muscles and pelvic girdle. From a cardiological point of view three patients presented sudden death and three others have a pacemaker. The genetic analysis was performed in genomic DNA of the patients and relatives. All patients showed a frameshift mutation in exon 1 of *LMNA* gene in heterozygous state: *c.240delG, p.Ala80Ala_fsX16*. The mutation has not been previously described and 180 control chromosomes were analysed in order to exclude a polymorphism. The analysis of different markers in *LMNA* locus as well as the absence of mutation in desmin gene (*DES*) shows that the disease is caused by the *LMNA* mutation.

Four out of the eight fourth generation children presented the mutation although they remained asymptomatic at the moment of the molecular study. The genetic diagnosis is useful to offer close cardiological monitoring of the patients in order to prevent a possible sudden death. Moreover the detection of the mutation allows for genetic counselling in the family.

P02.107 First deletion mutation in *SPRED1* in Legius syndrome

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The proband was born as a second child of healthy, non-consanguineous Caucasian parents at week 35 because of mothers diabetes mellitus. Birth measurements were 4035 g/ 51 cm/ 34 cm. As a newborn ptosis of her left eye was noticed. She was initially referred to a clinical geneticist at the age of 14 months for evaluation of multiple small cafe au lait macules (CALM). At the age of 4 years she had 6 CALM over 1 cm in diameter and several smaller ones and ptosis of the left eye. She had no freckling, no neurofibromas and no iris Lisch nodules. Her brain MRI, speech and motor development are normal. In ophthalmological follow ups her vision has been normal.

Her 7-year-old brother has 10 CALM over 1 cm, flat feet, no freckling, no neurofibromas, no Lisch nodules, a normal brain MRI, normal milestones, and mild learning difficulties. The 41-year old father has 6 CALM larger than 1.5 cm, a few xanthelasmas under the eyelids, no freckling, no neurofibromas, no Lisch nodules and his brain MRI was normal.

Comprehensive mutation analysis of *NF1* was negative. Because of multiple CALMs *SPRED1* mutation analysis was performed but no mutation was found by direct sequencing of the entire coding region. Further analysis for presence/absence of copy number changes using MLPA, qPCR and aCGH showed presence of a deletion of the first exon and the promoter region in *SPRED1* in this family. This report shows the importance of copy number studies in Legius syndrome.

P02.108 Identification of mitochondrial mutations in LHON patients

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LHON (Leber's hereditary optic neuropathy) is a maternally inherited disease typically leading to acute bilateral blindness due to loss of optic nerve and papillomacular bundle nerve fibers, predominantly in adult males with prevalence estimated to be 1:50,000. Many factors like mtDNA background, heteroplasmy of mtDNA mutation, nuclear genes, and environmental factors, have been shown to play active roles in pathogenesis of LHON. It has been well established that optic atrophy is a very common and sometimes the singular pathological feature in mitochondrial disorders. We studied the prevalence of primary LHON mutations and other mitochondrial abnormalities by sequencing the entire mtDNA coding region. 10 LHON diagnosed cases were enrolled for this study. DNA was extracted from whole blood samples and PCR were done for coding region of the mitochondrial genome. All fragments were sequenced in both forward and reverse directions for confirmation of any detected variant. Total 31 variants were found in this study, 12 (38.70%) were nonsynonymous and 19 (61.30%) were synonymous. Our results imply a broader association between potentially pathologic mtDNA sequence changes and severity of optic nerve injury in LHON. As the percentage of mutant mtDNAs increases, mitochondrial energy production declines and ROS increases. Increased ROS acts as a mitogen, but excessive ROS together with reduced energy production can lead to apoptosis. We report a fairly small group of patients from a restricted ethnic population and highlight the need for analysis of large number of samples to identify primary causal mutations in Indian population.

P02.109 Ligneous conjunctivitis with severe ligneous periodontitis and decreased serum plasminogen: the first Egyptian case report.

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Ligneous conjunctivitis (MIM 217090) is a rare autosomal recessive hereditary disorder. We report a 12 year old girl with dysmorphic features with both **ligneous periodontitis** and **ligneous conjunctivitis** in association with plasminogen type I deficiency. Intra-oral examination revealed remarkable gingival enlargement, with pinkish, waxy, painless masses. The gingival papillae were hyperplastic concealing most of the teeth. Areas of the gingival tissues were covered with a dull and rather tough whitish yellowish membrane. A thin pseudomembrane that could be wiped away overlay the tough part of the membrane. Ophthalmic examination revealed mild swelling of both eye lids, white pseudomembranes on the upper and lower tarsal conjunctivae with slight involvement of the bulbar conjunctivae and scarring of the tarsal conjunctivae. Diagnosis was based on the clinical and histological findings and most importantly, decreased serum level of plasminogen type I.

P02.110 A French experience of limb malformation gene analyses: stringent clinical selection guarantees better percentage of mutation identification.

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Our objective was to evaluate the consequences of accurate clinical selection in the mutation identification for genes implicated in limb development

Patients: 403 DNAs from index patients (30% familial) were sent to our laboratory with a diagnosis and a request for molecular analyses.

Methods: For each syndrome, we classified the cases as "typical", "atypical" or "excluded". 451 PCR-sequence of exonic and flanking intronic regions as well as MLPA or Q-PCR were performed for each analysed gene (133 *TBX5*, 83 *SALL4*, 69 *TP63*, 47 *SALL1*, 37 *LMX1B*, 28 *CDMP1*, 22 *ROR2*, 13 *TBX3*, 11 *IHH*, 4 *TBX4*, 4 *ZRS*).

Results : 192 cases were considered "typical", 164 "atypical" and 47 "excluded".

139 (34.5%) deleterious anomalies were identified. 134 among "typical" (70%), 5 among "atypical" (3%), and none among "excluded" cases. In the atypical group referred for Holt-Oram syndrome (HOS), four cases were mutated for *SALL4* and one carried a *TBX3-5* deletion. Further tests (Array-CGH or molecular analyses) lead to a diagnosis in 22 additional cases. The final diagnostic rate was 53%.

The mutation rate differed among diseases: 95% Nail-Patella patients were considered "typical", 83% of whom were *LMX1B* mutated. When typical (47 %), 81% HOS patients were carrying *TBX5* mutation. *CDMP1* mutation rate reached 94% in typical patients with type C brachydactyly or Grebe syndrome. *TP63* associated diseases were more difficult to diagnose (41% typical) and a mutation was identified in 46%.

Conclusion: an accurate clinical diagnosis allows improving mutation detection rate for limb malformation genes.

P02.111 Congenital Muscular Dystrophy associated with a de novo LMNA variant: Pathogenic mutation or polymorphism?

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Aims: To describe a newly emerging congenital muscular dystrophy phenotype associated with a de novo LMNA variant

Methods: Extensive genetic, histological, neurological and radiological investigations were undertaken.

Results: A term baby, born with bilateral severe positional talipes, was investigated after initial respiratory distress, developed an oxygen requirement and was eventually ventilated due to progressive respiratory muscle weakness. Central hypotonia was noted initially, but then was shown to have absent reflexes and profound axial hypotonia yet milder peripheral hypotonia and muscle weakness. There was facial weakness as well. There was progressive right atrial enlargement on echocardiogram. Muscle CK was 1243 U/L, brain MRI and nerve conduction studies were unremarkable, muscle biopsy showed only mild myopathic change. LMNA mutation analysis identified a de novo heterozygous change that is likely to be associated with the severe presentation. This patient died after developing seizures and interstitial pneumonitis as an end stage event.

Conclusion: LMNA mutations can cause a severe congenital muscular dystrophy that have been hitherto under recognised. Functional studies and further reports of a similar presentation with the same variant may help provide proof of pathogenicity. Awareness of this phenotype may allow increasing diagnosis of this rare presentation in the future.

P02.112 Loeys Dietz syndrome: clinical and molecular characterization of 4 Spanish patients

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Introduction: Loeys-Dietz syndrome (LDS) is characterized by vascular and skeletal manifestations, with aggressive arterial aneurysms and high incidence of pregnancy-related complications. Autosomal dominant inheritance pattern with clinical variability. No genotype-phenotype correlation.

Objective: Clinical and molecular characterization of four patients with LDS.

Results: Three patients are members of the same family and the fourth patient is an isolated case. All of them were sent to consultation with Marfan syndrome diagnosis. All of them have aortic root dilatation, with progressive course. Two of them required surgery. One patient also has dilatation of intracranial arteries. All of them have marfanoid-like habitus with no true dolichostenomelia, severe scoliosis, and normal ophthalmological evaluation. Two of them have bifid uvula. Two patients have atopic dermatitis. TGFBR2 mutations have been identified in both families (one with autosomal dominant inheritance-c.1273A>G exon 5 (p.M425V); and the other case is de novo-c.1583G>T exon 7 (p.R528H)). All patients have been sent for strict follow up by cardiologist, total body angioMRI, and given risk assessments of pregnancy. Conclusions: 1) Loeys Dietz syndrome must be considered in the differential diagnosis of Marfan syndrome. 2) An early diagnosis is important due to the natural history of this condition (worse prognosis of aortic root dilatation-dissection, risk of uterine rupture during pregnancy etc). 3) Clinical variability is present in the family described suggesting other modifying genetic factors. 4) The identification of mutations in TGFBR2 in our patients allows adequate genetic counselling. 5) To our knowledge this is the first report of LDS in Spanish patients.

P02.113 Mitochondrial 4917 A>G Mutation in a Iranian Family with Long QT Syndrome (LQTs)

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LQTS are inherited or acquired disorders of repolarization identified by the electrocardiographic (EKG) abnormalities of prolongation of the QT interval corrected for heart rate (QTs) usually above 460 -480 ms. Several reports indicate a relationship between mitochondrial DNA mutation and heart disorders. We identified a homoplasmic 4917 A>G mutation in the Mitochondrial ND2 gene in 14 members of a family with Long QT syndrome by PCR-SSCP. This Mutation causes a change of Asn to Asp (N150D Missense mutation). In this study, the effects of this Missense mutation upon transmembrane helixes were assayed by means of SOSUI System (transmembrane helix prediction). The result of this prediction showed that N150D mutation caused to decrease nine to eight transmembrane helixes. Thus, this mutation might trigger syncope or sudden death with emotional stress and exercise.

Key words: Long QT syndrome, mt DNA, Muration, SSCP.

P02.114

Madelung's deformity in a girl with a novel GNAS mutation

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Madelung's deformity is an uncommon congenital anomaly of the wrist. It is characterized by a decreased carpal angle with a triangular arrangement of the carpal bones, a shortened and curved radius, a widened distal radioulnar joint and a posterior displacement of the ulna head. The deformity is progressive and usually seen in adolescent girls. Although some asymmetry can occur, the anomaly is typically bilateral and symmetrical. The presumed cause is a partial early fusion of the ulnar side of the radial epiphysis. Turner syndrome and Leri-Weill dyschondrosteosis are disorders most commonly associated with Madelung's deformity. Here we present a 14-year-old girl with a Madelung's deformity, mild mental retardation, some dysmorphic facial features and a relative short stature. She also has a brachydactyly type E, with brachymetacarpia and brachytelephalangy. Standard chromosome analysis, array-CGH and analysis of the *SHOXab* gene showed no abnormalities. However, we did identify a novel and de novo mutation (c.476T>C; p.Val159Ala) in exon 6 of the *GNAS* gene,

the gene associated with Albright hereditary osteodystrophy. To our knowledge, this is the first report of Madelung's deformity in a patient with a *GNAS* mutation.

P02.115 Mandibulofacial dysostosis with microcephaly, cleft palate, and anomalous ears

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Mandibulofacial dysostosis (MFD) is an inborn error of craniofacial development affecting structures derived from the first and second branchial arches. Treacher Collins syndrome is the prototypical form of MFD, however, MFD is also a feature of several other syndromes whose genetic basis remain largely unknown. Two previously published reports describe seven patients with a novel MFD phenotype characterized by microcephaly, cleft palate, and anomalous ears (Guion-Almeida et al, Clin Dysmorphol 15(3):171-4; Wieczorek et al, Am J Med Genet A 149A(5):837-43); we believe these reports describe the same phenotype, and have provisionally termed this condition 'MFD with microcephaly' (MFDM). Building upon these earlier reports, and incorporating five additional cases, we present an updated clinical description of the MFDM phenotype. Cardinal features of this condition are short stature, developmental delay, microcephaly, metopic craniosynostosis, midface hypoplasia, micrognathia, and cleft palate. A highly specific ear phenotype is also observed, characterized by small, low-set ears, preauricular tags, auditory canal stenosis, and hypoplastic lobe/tragus. Other, inconsistently observed anomalies include cardiac septal defects, polydactyly, choanal atresia, and epilepsy.

We are presently studying the molecular basis of this condition. TCOF1 mutations have been excluded in several probands, confirming that MFDM is both genetically and clinically distinct from Treacher Collins syndrome. Genomic microarray delineated a complex de novo rearrangement in one proband; this individual has a microdeletion of an agenic region of 4p12, in conjunction with a deletion/duplication situated at 17q21.31. The latter region contains a number of appealing positional candidates for the causative gene underlying this condition.

P02.116 Molecular studies in patients with marfanoid features

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The Marfan Syndrome (MFS) is an autosomal dominant disorder caused by mutations in the fibrillin-1 (FBN1) gene. Ghent criteria are commonly followed for the clinical diagnosis of MFS.

The purpose of our study is to evaluate the diagnostic approach to MFS. We have analyzed the medical histories of 154 patients with "marfanoid features" referred to the Marfan multidisciplinary clinic. The results are shown in tables 1&2.

Several observations are relevant: Altogether 5 "new" mutations have been found, 2 in the group not fulfilling the Ghent criteria (MF-like). On the other hand, 3 cases with mutation in the TGFBR1 gene were detected, thus confirming the suspicion of the Loeys-Dietz Syndrome. In addition, 7.8% of our confirmed MFS cases would not have a diagnosis of MFS if molecular analysis had not been performed.

We conclude that in patients with "marfanoid features", molecular studies are relevant to confirm or sometimes change our clinical diagnosis, and particularly for reproductive counseling and evaluation of relatives at risk. Finally, our experience contributes to expanding the clinical phenotype of FBN1 and other genes' mutations, and may elicit minor revisions to the current Ghent criteria.

Table 1: Yield of molecular studies in our series of patients with marfanoid features

| Total number of patients referred | Clinical diagnosis | | Molecular study performed | Result | |
|-----------------------------------|--------------------|---------|---------------------------|----------|------------|
| | MFS | 87 | | FBN1+ | 55 (81%) |
| 154 | | Pending | 68 | Pending | 7 (10%) |
| | | | | Negative | 6 (9%) |
| | MF-like | 36 | 22 | FBN1+ | 2 22.7% |
| | | | | TGFBR1 | 3 |
| | | | | Pending | 5 (22.7%) |
| | | | | Negative | 12 (54.5%) |

Table 2: Evaluation of clinical data (following Ghent Nosology) after molecular diagnosis of FBN1+

| FBN1+ | Only 1 > criterion and involvement of other organs/systems | Family history (2 nd > criterion) | |
|----------------|--|---|------------|
| | | positive | negative |
| 57 index cases | 15 | Cardiovascular 7 Skeletal 6 Ophtalmological 2 | 8 7 (7.8%) |

P02.117 The case report of causal mutation of neonatal Marfan syndrome

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Neonatal Marfan syndrome is a rare and serious illness of connective tissue with atypical features of Marfan syndrome. It is a hereditary disease, but its neonatal form has a spontaneous mutation tendency. Neonatal Marfan syndrome also differs from classical form not only in certain symptoms, but in higher mortality rates during young age. Life span of patients with neonatal Marfan syndrome is less than 2 years, and it depends on the severity of cardiovascular complications.

Mutation analysis of neonatal form is different from the classical form, which is due to mutations in all 65 exons of the gene *FBN1*. Screening is associated with „neonatal region“ of the *FBN1* gene, that includes exons 24 to 32. Direct sequencing of these exons allows quick detection of mutation.

We described the case of this rare form of Marfan syndrome. Our patient after birth showed a clear phenotype: tall stature, thin body build, long arms, legs, fingers and toes and other symptoms typical for neonatal Marfan syndrome.

Based on the patient's phenotype without family history, we have decided to do molecular genetic analysis of this region of *FBN1* gene. We detected mutation in exon 29 and we confirmed the neonatal Marfan syndrome. Heart failure was the reason of death of our patient in the second month after birth.

P02.118 Marshall syndrome in a nine-year-old Turkish girl

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Marshall syndrome is a rare, autosomal dominant trait which is characterized by ocular abnormalities, early sensorineural hearing loss, high myopia and a distinct craniofacial anomalies.

We present a nine-year-old girl who was the second child of a non-consanguineous couple. She was referred to our department for dysmorphic appearance. Her karyotype was found 46,XX.

She was diagnosed with Marshall syndrome based on the clinical features of a depressed nasal bridge, anteverted nares, hypertelorism, short stature, micrognathia, high palate, and ophthalmologic anomalies. Ophthalmologic examination revealed cataracts, strabismus and severe degenerative myopia. Audiologic test showed sensorineural hearing loss. Her mother had osteoporosis, sensorineural hearing loss and severe myopia. It was evaluated the result of decreased penetrance.

Marshall syndrome proceeds variable ocular, otologic and skeletal symptoms so these patients require regular follow up to insure early detection of problems.

P02.119 MECP2 gene duplication in a Czech family with four affected boys

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Duplications of a part of Xq28 involving the methyl CpG binding protein 2 (MECP2) gene have been described in male patients with severe mental disability, delayed milestones, absence of language, hypotonia replaced by spasticity and retractions, and recurrent severe infections. We present a family with four affected boys in two generations. Three of the boys died in childhood due to respiratory infections. The proband, an eight-year-old boy, was found to carry a submicroscopic duplication of approximately 1.25 Mb in Xq28 including the MECP2 gene. He suffered from severe mental retardation, epilepsy and autistic features. The duplicated region contained about 56 protein-coding genes. These included 13 genes where loss-of-function mutations are known to cause X-linked disease. The MECP2 gene is essential in neuronal development, and is the only gene in the aberration of our patient where duplications have a known phenotypic effect. The mother of the patient, his maternal grandmother and aunt also carried the duplication. All of them were asymptomatic. The mother and the maternal grandmother of the patient showed skewed X-inactivation (15:85 and 7:93, respectively). In addition, the proband and his mother, but not the maternal grandmother and aunt, carried a duplication of the terminal 0.5 Mb of Xp. This de novo aberration most likely exerted no phenotypical effect, in accord with other published cases, and the phenotype observed in the family was most likely attributable solely to the duplication of the MECP2 region. This work was supported by grants MZOFNM2005 and CHERISH (EC FP7 223692).

P02.120 Duplication of Xq28, including MECP2, in a boy with the de novo cryptic unbalanced t(X;6)(q28;q27) translocation

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Duplication of the distal long arm of the X chromosome is a rare chromosomal abnormality. It may result from an intrachromosomal duplication or an unbalanced translocation with an autosome or with a Y chromosome.

We report a case of *de novo* duplication of Xq28-qter resulting from translocation onto the long arm of chromosome 6 in a boy with severe developmental delay, microcephaly, axial hypotonia, spasticity of upper and lower limbs, seizures, and severe recurrent infections. Apart from an open mouth and protruding tongue no other specific dysmorphic facial signs were seen. Feeding difficulties with poor sucking, generalized hypotonia and cryptorchidism were observed during the neonatal period. Prader-Willi syndrome was excluded in differential diagnosis. Routine cytogenetic analysis results were normal. Using FISH, MLPA, and array CGH (Affymetrix Whole Genome 2.7M Array), we identified and characterized a terminal duplication of chromosome X at q28-qter (approx. 3.346 Mb in size) involving MECP2 and a 6q terminal deletion with the breakpoint in q27 (approx. 1.89 MB). This is the second report of a boy with a cryptic unbalanced Xq-autosome translocation.

Subtelomeric 6q27 deletion due to relatively mild phenotypic presentation is difficult to recognize clinically. Disomy for the Xq28 chromosome region yields a more characteristic phenotype, however, so that a clinically oriented FISH study using subtelomeric probes or MLPA can easily detect a cryptic rearrangement.

We strongly recommend the use of MLPA as the first screening method for the detection of copy number aberrations in such patients because of its cost-effectiveness.

P02.121 Refining the phenotype associated with MEF2C haploinsufficiency

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Recently, submicroscopic deletions of the 5q14.3 region have been described in patients with severe mental retardation, stereotypic movements, epilepsy and cerebral malformations. Further delineation of a critical region of overlap in these patients pointed to MEF2C as the responsible gene. This finding was further reinforced by the identification of a nonsense mutation in a patient with a similar phenotype. In brain, MEF2C is essential for early neurogenesis, neuronal migration and differentiation. Here we present two additional patients with severe MR, autism spectrum disorder and epilepsy, carrying a very small deletion encompassing the MEF2C gene. This finding strengthens the role of this gene in severe MR, and enables further delineation of the clinical phenotype.

P02.122 Melnick-Fraser Syndrome In Four Generations

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Brancio-Oto-Renal (BOR) Syndrome is a rare autosomal dominant disorder. The association of branchial arch anomalies (Ear malformations, preauricular pits, branchial fistula), hearing loss and renal hypoplasia. The hearing loss is variable conductive, mixed forms, and sensorineural cause usually in lower frequencies from 20 to 100 db. The more severe disiasi, the greater degree of hearing loss patients have. Renal anomalies have included: bilateral renal dysplasia, bilateral polycystic kidneys and various malformations of collecting system which end to chronic renal failure. We are reporting four generations with different type of renal anomalies and hearing loss in a large family with 14 affected patients. They have variable expression of BOR syndrome, which is confirmed by detection of EYA1 gene. For one of families we did prenatal diagnosis because they had two affected offspring.

P02.123 Duplications of 17q12-q21 in patients with mental retardation, growth disturbances and facial dysmorphism

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Purpose: In recent years, small genomic rearrangements of 17q12 and 17q21.31 have been described in patients with autism, cognitive impairment/mental retardation, renal disease, epilepsy, and/or growth retardation. We present three unrelated patients with partially overlapping duplications in the 17q12-q21.32 region.

Methods: Microarray analysis (Affymetrix SNP_6.0, BAC array, or oligo array) was performed on purified DNA from peripheral lymphocytes. FISH analysis was done on cultured lymphocytes according to standard protocols.

Results: We identified three unrelated patients with partially overlapping *de novo* duplications in the 17q12-q21.32 region. All patients were investigated with genome wide array platforms, showing sizes of the duplications of 1.5 Mb, 6.6 Mb, and 7.7 Mb respectively. Though the patients had diverse clinical features as well, all presented with mental retardation, facial dysmorphism and growth disturbances.

Conclusion: We report three patients with mental retardation, growth disturbances and facial dysmorphism carrying partially overlapping duplications of the gene-rich locus on chromosome 17q12-q21.32. We add new clinical features to the variable phenotype of 17q12-q21.32 duplications.

P02.124 Screening for common submicroscopic deletions /duplications in Bulgarian MR/DD patients

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Introduction: Since the discovery that humans possess 46 chromosomes and association of different aneuploidies with specific phenotypes karyotyping was rapidly introduced into workup of patients with mental retardation (MR) and birth defects. With the introduction of fluorescence in situ hybridization the detection of submicroscopic chromosomal imbalance became possible. Methods as MLPA, QF-PCR and array based CGH blur the boundaries between karyotyping and molecular genetics. Submicroscopic deletions and duplications are observed in at least 3-5% of patients with variable degree of MR/DD. MLPA is shown to be a rapid, inexpensive alternative of FISH, allowing simultaneous screening of multiple specific submicroscopic deletions/duplications.

Material and methods: A cohort of 30 preselected patients with varying degree of developmental delay or MR was screened for subtelomeric imbalances using MLPA.

Results: In this pilot study we started screening for known disease related microdeletion/duplications of our patients using MRC's MLPA kit P245-A2. In patients showing normal results additional testing for subtelomeric rearrangements using P070-B1 was performed. Patients were pre-selected by karyotyping and when needed by metabolic screening. Using P245-A2 we found 3 (10%) aberrations - 2 deletions and 1 duplication. Screening for subtelomeric regions revealed aberrations in 10 patients (43%) - 7 deletions and 3 duplications. High detection rate in our pilot study could be explained by the strong clinical selection of patients.

P02.125 CHERISH - Improving Diagnoses of Mental Retardation in Children in Eastern Europe and Central Asia through Genetic Characterisation and Bioinformatics/Statistics

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The CHERISH consortium, funded by EU FP7 under grant agreement #223692, is made mainly of Eastern European countries. It aims at creating a large collection of patients affected with developmental delay (syndromic and non-syndromic). The project will enhance the diagnostic possibilities for these patients and gather epidemiologic data on the genetic causes of mental retardation in Eastern Europe. General information and updates can be retrieved through <http://www.cherish-project.eu/>.

During the first year of the project, partners started the collection of clinical data using a specific questionnaire. So far, data of more than 400 patients have been uploaded on the dedicated database. Search of cryptic chromosome rearrangements is carried out through array-CGH or SNP-CGH analysis in the laboratories of University of Bologna and University of Tartu. Candidate genes from aberrant regions will be sequenced in patients with similar phenotypes. Families with X-linked mental retardation are being analyzed, on a first approach, through an all-exons X-chromosome array, available in the Cyprus Institute of Neurology and Genetics, while the laboratory of Poznań University of Medical Sciences will analyze specific genes on the X chromosome for patients with normal array results. In sporadic cases with normal array analysis and in families with apparent autosomal dominant inheritance, sequence analysis of the SYNGAP1 and STXBP1 genes is performed in Bologna. Special attention is dedicated to siblings affected with mental retardation born from consanguineous parents, who are suitable for homozygosity mapping. The final results on the first cohort of up to 100 families will be discussed in details.

P02.126 Multiplex ligation-dependent probe amplification (MLPA) analysis of subtelomeric chromosome rearrangements in individuals with idiopathic mental retardation.

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INTRODUCTION: Described in approximately 5% to 7% of individuals with unexplained MR, subtelomeric rearrangements have emerged as a significant cause of both idiopathic and familial mental retardation. The Multiplex Ligation-dependent Probe Amplification (MLPA) technique has been considered as a suitable alternative to identify these anomalies. OBJECTIVE: To investigate the contribution of subtelomeric rearrangements among the causes of idiopathic MR using the MLPA technique. METHODS: 141 unrelated patients were selected based on clinical criteria; all of them had previously been subjected to karyotyping and molecular analysis for fragile X syndrome. DNA samples were extracted and purified from peripheral blood leukocytes. They were tested using specific subtelomeric MLPA kits SALSA P036-E1 and P070-A2 HUMAN TELOMERE according to manufacturer's instructions. The amplification products were separated by capillary electrophoresis using an ABI PRISM 310 automated sequencer and size standard. MLPA data were extracted and analysed by GeneScan®, Genotyper® and specific Microsoft Excel® spreadsheet for each kit. RESULTS: Until now, 91 patients with idiopathic MR were analyzed and four subtelomeric deletions/duplications were identified (4.4%): one case with a 1p36 deletion and one with a 6p deletion, besides two cases with a combined deletion/duplication, involving 5p/9p and 4p/12p regions, respectively. These subtelomeric rearrangements had not been previously identified by conventional technique and FISH tests are being provided in order to confirm them. CONCLUSION: Subtelomeric rearrangements must be investigated in individuals with unexplained MR and MLPA is a rapid and effective technique for detecting these abnormalities.

P02.127 A New Case of Metaphyseal Acroscyphodysplasia

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Metaphyseal acroscyphodysplasia (MASD), also known as Bellini's metaphyseal dysplasia, is a particularly rare skeletal dysplasia with presumed autosomal recessive inheritance. So far, no disease-genes have been identified. The main clinical features of MASD are disharmonic dwarfism, micromelia predominating in the lower limbs, severe brachydactyly, cranio-facial dysmorphisms, scoliosis, flexion contracture of knees, and mental retardation. The diagnosis is based on typical radiographic findings such as cupped metaphyses ('scypho-' comes from the word 'scyphus', meaning cup) and cone-shaped epiphyses of long bones, especially appreciable at the knees, coxa valga, genua vara, short and broad femurs, tibial bowing, mild humeral shortening, short metacarpals, metatarsals and phalanges, and advanced bone age.

We report the case of a 12-year-old boy with clinical and radiological features that are consistent with the diagnosis of MASD (dwarfism, short limbs with marked acromelic shortness, coxa valga, cup-shaped metaphyses and cone-shaped epiphyses of knees, flexion contracture of knees, cranio-facial dysmorphisms, and mental retardation). The family history was negative for mental retardation, short stature and other signs suggestive of skeletal dysplasia. Although the parents denied consanguinity, they were both born in the same small town, which counts only 11,000 inhabitants. So far this is the 14th case of MASD described in the literature.

P02.128 Micro syndrome: report of a two-year old boy

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Micro syndrome is a rare, severe autosomal recessive disorder and characterized by microcephaly, microcornea, congenital cataract, mental retardation, optic atrophy, and hypogenitalism. Third child of a consanguineous couple was referred to our clinic because of facial dysmorphism and neurodevelopmental delay. Two death children in this family were also thought to be affected. Physical examination of 2-year-old boy revealed that he had microcephaly, hypertrichosis on his forehead, prominent nasal root, thin and overhanging upper lip, short philtrum, large prominent ears, high arched palate, a pointed chin, contracture at the hands, obesity, mental-motor retardation and hypotonia. His weight was 15 kg, length 88 cm(25th centile), and OFC 47 cm (10th centile). Chromosomal analysis showed a normal 46,XY karyotype. He had congenital hypothyroidism.

Ocular findings are the most reliable diagnostic findings of Micro syndrome. Our patient had ophthalmologic signs include: microphthalmia, microcornea, congenital cataract, bilateral mild ptosis, and severe cortical vision impairment. Cataract surgery was performed at 7th month. Hypogenitalism is an another major feature of this syndrome. This case had micropenis, cryptorchidism and small scrotum. Urether dilatation and bladder diverticulum detected by ultrasonography(US). His cryptorchidism confirmed by US,too

Neurological examination showed hypotonia. He had no seizures but electroencephalogram revealed left hemisphere asymmetry with cerebral bioelectric dysfunction. Brain MRI showed enlarged lateral and third ventricles, hypoplasia of corpus callosum and cerebellum in both hemispheres and hypotrophy of the white matter. The symptoms and features of our case compared with the previous Micro syndrome cases in literature.

P02.129 How a non-compaction left ventricle detect a monosomy 1p36 syndrome

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Aim: To present how a 1p36 monosomy syndrome was detected, due to a cardiac examination, after many years of neurologic follow up. Our patient, a 10 yo. boy, was treated for recurrent seizures; he associated right small cerebral cavernoma and arachnoid cyst. From birth he had distinct facial features and intellectual disability.

Material and methods: This patient was sent for a cardiologic examination due to a muscular hypotrophy. We performed clinical examination, ECG, Echocardiography and angio MRI. A genetic examination was done.

Results: No cardiac murmur and normal ECG was registered. Non-compaction of the left ventricle (NCLV), with deep trabeculations and deep inter-trabecular spaces were detected in apical, mid ventricular inferior and lateral wall; normal ejection fraction. Angio MRI confirmed the diagnosis. The laboratory tests were normal, so a muscular pathology was excluded. Genetic examination mentioned a normal karyotype. In the context of particular face, with deep-set eyes, straight eyebrows, midface hypoplasia, flat nose and associated cardiomyopathy, mental delay, seizures and cerebral tumors, an extended FISH test was performed. The suspicion of monosomy 1p36 syndrome was confirmed.

Conclusions: Muscular hypotrophy in a patient with particular face and complex neurologic modifications conduct to a cardiac exploration, because a muscular disease was suspected. Left ventricular non-compaction was detected; it is a rare cardiomyopathy, present in muscular disease, which were excluded and in other genetic syndromes. In the clinical context, a monosomy 1p36 syndrome was suspected and confirmed by FISH. NCLV when present, the genetic involvement has to be searched.

P02.130 C677T and A1298C mutations in methylenetetrahydrofolate reductase gene in mothers of children with trisomy 21

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Introduction. Deficiency of methylenetetrahydrofolate reductase (MTHFR) causes a deficiency of the active metabolic form of folic acid. This deficiency is cited as a promoting factor of chromosomal non-disjunction in gametogenesis, with increased risk for the appearance of aneuploidies, therefore folic acid therapy could interfere with their prophylaxis. The study aims to evaluate the prevalence of C677T and A1298C mutations in MTHFR gene for mothers of children with trisomy 21.

Patients and methods. The study group consisted of 58 mothers of children with trisomy 21, ages between 18- 47 at the time of gestation, who were registered at the Genetic Pathology Centre of the First Paediatric Clinic, in the range 2008-2009. DNA analysis was performed using a PCR-RFLP technique.

Results. The two mutation prevalence (the evaluated group, in comparison with other studies), is presented in the table below:

| Mutation | The present study | Stuppia L. (Italy,2002) | Acacio G.L. (Brasil,2005) | Kohli U. (India,2008) |
|------------------------------|-------------------|-------------------------|---------------------------|-----------------------|
| Homozygous C667T | 8.6 % | 44 % | 7.1 % | 0 |
| Homozygous A1298C | 13.7% | | 4.3 % | |
| All Homozygous | 22.3% | | 11.4 % | |
| Compound heterozygous | 18.9% | | 27.1 % | 28 % |

Conclusions. Our study, a priority on a national scale, reveals important differences regarding the prevalence of the two mutations, in comparison with studies conducted on other population groups. For a reliable assessment of their role, studies conducted on larger groups compared to control groups are necessary.

P02.131 Myhre syndrome: report of three unrelated patients

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Myhre syndrome (MS) is a very rare condition mainly characterized by short stature with rhizomelia, muscular hypertrophy in males, a non-progressive joint stiffness, mixed deafness, hypertension and a peculiar facial appearance consisting in midfacial hypoplasia, short philtrum, thin lips and prognathism. Sixteen sporadic cases (11 males and 5 females) have been reported so far. The higher predominance of males and the milder manifestation in the females suggested an X-linked recessive inheritance, even if a de novo dominant mutation is still a possibility. Here we describe 3 unrelated patients (2 females and 1 male), fitting the clinical diagnosis of MS. One female patient presented megacolon, whilst the male developed multiple neoplastic lesions. Both these striking clinical findings haven't been previously reported in this condition, allowing to expand the clinical phenotype of MS. Moreover we performed array-CGH analysis in the patients, but it gave normal results in all of them.

P02.132 Myotonia congenita (MC) in Russia: the two frequent mutations in CLCN1 gene allows to reveal 14% mutant chromosomes of patients with MC.

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Myotonia congenita (MC) is a hereditary muscle disorder characterized by delayed relaxation of skeletal muscle after voluntary contraction (myotonia). MC is caused by mutations in the skeletal muscle chloride channel gene CLCN1 (7q35).

The study group was consisted of 61 unrelated patients (12 dominant, 9 recessive and 40 sporadic cases) with myotonia revealed by clinical examination. We analyzed all DNA samples by direct sequencing of exons 3, 8, 11, 12, 13, 14, 15, 16, 21, 22 of CLCN1 gene and detected

16 different mutations for 29 patients (21 patients had one mutant allele and 8 ones had two mutant alleles). Seven of them have not been described earlier (c.912A>C, c.1024C>T, c.1699A>C, c.1679T>C, c.1720C>A, c.2555_2558delCCTT, c.2284+9_2284+10insg). Other mutations were brought in mutation data bases (Leu198Val, Thr268Met, Phe413Cys, c.1447_1450del, Ivs13+1G>A, Ala493Glu, Tyr686Stop, Ivs18+5C>T, Ivs19+2T>A). Two of these mutations were detected in several unrelated patients: c.1436_1449del in exon 13 of *CLCN1* gene and Ala493Glu in exon 14 of *CLCN1* gene. The c.1436_1449del in exon 13 was detected at the compound heterozygous state in 6 sporadic cases and in 3 recessive cases and in 1 dominant case. The Ala493Glu in exon 14 was detected at the compound heterozygous state in 3 sporadic cases and in homozygous state in one recessive case. The c.1436_1449del and Ala493Glu mutations were found in 29 % and 13 % accordingly of all mutant chromosomes. Diagnostics only two these mutations allows to reveal 14% mutant chromosomes of patients with MC. The investigation is in progress now.

P02.133 Clinical and molecular findings of an Italian family with atypical myotonic dystrophy type 1 (DM1) associated with a CCG repetition in the 3'UTR of the DMPK gene

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Myotonic Dystrophy type 1 (DM1, MIM #160900) is the most frequent form of adult muscular dystrophy and is caused by a CTG expansion located in the 3' UTR of *DMPK* gene. It is a multisystemic disorder characterized by dystrophic changes of muscles, electrical or clinical myotonia, bilateral cataract, and variable involvement of other tissues and organs, including central nervous, cardiac, gastrointestinal, endocrine and immune systems. In some patients, other symptoms may be present, but the causes for this widely variable expression are still largely unknown. Here, we describe the clinical and molecular features of a large Italian family whose affected members show an atypical form of DM1, manifesting in adult age, with encephalopathy, aspects of neurogenic atrophy of muscles, pes cavus, deafness and variable features of classic DM1. Analysis of the *DMPK* gene revealed the presence of unusual molecular findings, with CCG repeats interspersed at the 5' and 3' portion of the CTG repeated region. This is the first description of patients with atypical DM1 associated with triplets repeats different from CTG. The contribution of the CCG repetition to the clinical phenotype and variable expression of DM1 in this Italian family is currently under investigation.

P02.134 Severe demyelinating polyneuropathy, sensorineural hearing loss, spasticity, vestibular dysfunction and stroke like episodes in homozygous myotonic dystrophy

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In 2009, a family with atypical myotonic dystrophy, characterised by intermediate Charcot-Marie-Tooth, sensorineural hearing loss and attacks of impaired consciousness with focal neurological signs, was reported by Spaans and colleagues. We report a 40 year old male with a similar phenotype.

His mother and maternal uncle have very mild late onset myotonic dystrophy type 1, both being heterozygous for a small expansion at the *DMPK* locus. There is no information about his father. Our patient developed progressive sensorineural hearing loss from childhood requiring hearing aids from age 10 years. Since the age of 14 years he suffered with episodes of vertigo and by age 30 had persistent vestibular impairment resulting in use of a wheelchair. Aged 39 he suffered two stroke-like episodes during which there was reduced consciousness, dysphasia, dysarthria and left hemiplegia following which he had residual left sided weakness. Examination aged 39 revealed lower limb spasticity with pes cavus and sustained clonus along with signs of peripheral neuropathy and left hemiplegia. Cerebellar signs and myotonia were absent. Neurophysiology confirmed a severe demyelinating sensorimotor neuropathy but myotonia was absent. Brain MRI showed non specific periventricular high signal and spinal MRI was

normal. Genetic testing of *PMP22*, *MPZ*, *GJB1*, *SCA1*, 2, 3, 6, 7, 17 and mitochondrial mutations m.3243A>G and m.8993T>C/G revealed no abnormality. Direct PCR at the *DMPK* locus revealed expansions of approximately 53 and 71 CTG repeats.

Our patient's clinical presentation will be discussed and compared to that reported in both atypical myotonic dystrophy and *DMPK* expansion homozygotes.

P02.135 Phenotypic variability in patients with deletions in the neurexin-1alpha gene

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Deletions in the neurexin-1alpha gene (NRXN1 α) have been identified in large-scale screens for copy-number variations in patients with autism or schizophrenia. In addition, homozygous deletions in the gene were recently described in a patient with Pitt-Hopkins like phenotype. Neurexin-1alpha codes for a cell-surface receptor that binds to neureolin and is associated with synaptogenesis and neurotransmission. Here we report 4 patients with NRXN1 α deletions, ranging in size between 110 and 400 Kb and located in the 5' end of the gene, identified by array-CGH. Three of them had unexplained learning difficulties and/or autism. In the first patient the deletion was inherited from his mildly affected mother and in the second the deletion had occurred de novo in addition to a de novo 16p11.2 deletion. The third patient was compound heterozygote with two different, partly overlapping deletions inherited from the healthy parents. This patient demonstrated a more severe phenotype. In addition, a deletion was identified in a newborn child with persistent neonatal hypoglycemia and feeding difficulties. Our data confirms previous findings and suggest that deletions of the neurexin-1alpha gene are highly associated with neurodevelopmental symptoms but with a very variable penetrance. Homozygous deletions are expected to cause a more severe phenotype and the recurrence risk for next pregnancy is in this family very high with two carrier parents.

The variable penetrance and the detection of a deletion in the NRXN1 α as a coincidental finding in a newborn child illustrate some of the difficulties in genetic counseling of array findings.

P02.136 Neurofibromatosis type 1 (NF1) and genetic diagnostic shown with a case

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Background: Neurofibromatosis type 1 (NF1) is a clinical diagnosis. NF1 includes symptomatology from different organ systems and anatomic localizations such as macrocephali, iris hamartomas, hypertelorism, glaucoma, hypertension, scoliosis, spina bifida, pseudoarthrosis, plexiform neurofibromas, café-au-lait spots, axillary and inguinal freckling, mental retardation, learning disabilities, hydrocephalus and different types of cancer.

Patients and families with NF1 are regularly referred to Telemark Hospital for diagnostics and genetic counselling.

Methods: We present a child with primary unknown diagnosis. The presentation illustrates genetic methods, FISH, mlpa and aCGH, used to diagnose NF1 in this child and the family.

Possibilities and limitations with todays diagnostics will be presented.

Results: Our results will be presented at the conference.

P02.137 Gonadal function in male patients with Nijmegen breakage syndrome, a cancer-prone disease with the DNA repair defect.

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Nijmegen breakage syndrome (NBS) is a severe chromosomal instability disorder, caused by hypomorphic mutations in the *NBN* gene, which product is critical for processing DNA double strand breaks. It is characterized by microcephaly, growth retardation, immune deficiency, and predisposition for malignancy. Due to diverse information on the reproductive function, depending on the *Nbs1* murine model, we investigated the course of puberty with respect to humans with NBS. Previously we presented hypergonadotropic hypogonadism in NBS females, however data on gonadal function in male patients are still limited.

The aim of the study was to evaluate sexual development along with hormonal assays in 18 NBS males (ages 1.17-25.92), homozygous for c.657_661del5 mutation, followed between years 1993 and 2008. They were divided in 6 subgroups, according to the age and pubertal Tanner stages. Concentrations of gonadotropins (FSH, LH) and testosterone were evaluated.

Puberty in NBS boys was initiated spontaneously and progressed similarly as in healthy peers. Gonadotropin levels in the prepubertal period were normal, whereas in older subjects the mean values tended to be slightly higher than the references ranges. Testosterone levels were normal in all groups.

Despite normal pubertal development in our group of NBS males, increasing gonadotropin levels in older patients may be indicative of gonadal dysfunction, which demands further supervision.

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P02.138 Family anamnesis with Nijmegen breakage syndrome with regard to the bronchial asthma

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Nijmegen breakage syndrome (NBS) is autosomal-recessive disease (homozygous carriage of 657del5 gene mutation NBS1) characterized phenotypically by microcephalia, "bird" face, combined immunodeficiency and susceptibility to malignant growths. It is the most spread in the Czech Republic, Poland and the Western Ukraine and carriers approximately 1% of population.

Purpose: to analyse genealogical peculiarities in families of patients (Ukraine) with NBS and cases of bronchial asthma in relatives of probands.

Patients and methods: Among 17 families there are four families (23,5%), in which NBS was diagnosed in two own siblings. In seven families (41,2%) the child with NBS was the only child. The clinical-genealogical method was used.

Results: In the 17 families, where a child with NBS was born, in 4 (23,5%) families bronchial asthma cases have occurred: from 1 to 4 cases. In the seven families, where a malignant tumor has developed in a child with NBS, there had been only one case of bronchial asthma (14,3%).

In the 10 families, where a child has been diagnosed NBS without oncopathology, the cases of bronchial asthma had been revealed in 3 families (30%) from 1 to 2 cases, which is by 2,1 times more frequent, than in the families, where a proband had had oncopathology. Probably, in such families the cases of bronchial asthma act as a protective mechanism with regard to the development of malignant tumors in a proband.

Conclusion: Medical-genetic consultation with the use of clinical-genealogical method is necessary for the families, where a child with NBS was born.

P02.139 Prevalence of the c.35delG and p.W24X Mutations in the GJB2 Gene in North-West Romania

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Objective: To determine for the first time in Romania the prevalence of the most frequent GJB2 mutations (c35delG and p.W24X) in healthy subjects and in patients with non-syndromic hearing loss (NSHL).

Material: 300 healthy adults (group A) and 75 unrelated children with NSHL (group B) from North-West Romania.

Methods: a. Group A : phonic acumetry and detection of the c.35delG (semi-nested-PCR, RFLP and ARMS-PCR analysis) and p.W24X (ARMS-PCR and RFLP analysis) mutations; b. Group B: audiological examination (otoscopy, tympanogram, acoustic otoemission and tonal audiogram or auditory evoked potentials) and detection of the c.35delG (semi-nested-PCR, RFLP and ARMS-PCR analysis) and p.W24X (ARMS-PCR analysis) mutations.

Results: Group A: The c.35delG mutation was present in heterozygous state in 11 cases (1.83% of the total 600 alleles examined), while the p.W24X mutation was absent. Group B: The number of reported mutation cases as against the number of alleles indicates a 33.3% frequency for c.35delG mutation and respectively 5.3% for p.W24X mutation. All 22 patients with 35delG/c.35delG genotype (19 patients), c.35delG/p.W24X genotype (2 patients) or p.W24X/p.W24X genotype (1 patient) presented profound/severe hearing loss.

Conclusion: Our study confirms that the frequency of the c.35delG mutation in healthy subjects and the c.35delG and p.W24x mutations in patients with NSHL from North-West Romania is comparable to that seen in other Central and South-Eastern European countries. The homozygous or compound heterozygous states represent a major risk factor for profound or severe deafness.

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P02.140 Systemic lupus erythematosus in a patient with Noonan syndrome and KRAS mutation

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Noonan syndrome is characterized by dysmorphic facial features, short stature and congenital heart defects. It is associated with coagulation factor deficiencies and thrombocytopenia. Development of Systemic Lupus Erythematosus (SLE) has been described as a rare complication. PTPN11, RAF1, SOS1 and KRAS are the responsible genes for the Noonan syndrome. PTPN11 mutations concern 50% of the patients.

The 11 year old female was referred to the clinic due to morphological stigmata and psychomotor retardation. The patient had short stature, a broad forehead, ptosis of eyelids, low set ears, curly hair and pectus excavatum. She also presented hypertrophic cardiomyopathy. After clinical evaluation DNA sequencing was performed. The mutation c77A>T, pAsn 26Ile was deleted in the KRAS gene. KRAS mutations are detected only in 2% of Noonan patients and this particular mutation has never been described before in the literature. Onset of Thrombotic Thrombocytopenic Purpura (TTP) associated with positive direct Coombs implied an immunological substrate which was confirmed by anti ds DNA. The TTP manifestations belonged to the SLE clinical spectrum.

Follow up is mandatory for the proper diagnosis of clinical signs and symptoms related to Noonan syndrome. Genetic testing should not be limited to the most frequent responsible gene PTPN11, because Noonan syndrome has high genetic heterogeneity

P02.141 A case of Noonan syndrome with late onset of bleeding disorder

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Introduction. Noonan syndrome (NS) is an inherited disorder characterized by typical various phenotypic features. Bleeding disorders are one of the most serious and common complications associated with NS.

Case report. A 17 years old male with typical Noonan features was admitted for a giant post-traumatic right buttock hematoma. He didn't

present bleeding tendency in childhood and at 7 years of age he had a heart surgical intervention without abnormal bleeding. At clinical examination splenomegaly was detected. Laboratory test show mild thrombocytopenia, with small platelet and reduced clot retraction, normal bleeding and clot time, prolonged APTT (40"), decreased prothrombine consume (25"), normal level of factors VIII, XI, IX, X, VII, V, vonWillebrand and decreased factor XIII (20%). He needed three surgical interventions to cure the hematoma: first with Novo Seven, second with Feiba - both with important bleeding despite of high doses of concentrate factors and the third intervention with Novo Seven, platelet concentrate and plasma with a good intra- and post- surgery control of hemorrhage.

Conclusions. In our case the bleeding disorder had a proved late onset and consists in a complex anomaly: thrombocytopenia with abnormal platelet function, factor XIII deficiency and possible von Willebrand disease with normal antigen and activity.

The existence of various types of bleeding disorders within one syndrome is unusual and the relation between abnormal bleeding and the underlying cause of Noonan syndrome is not clear. It is recommended to screen for bleeding disorders every patient with NS for improvement the clinical care of these patients.

P02.142 Increased lifetime risk of cancer in patients with Noonan Syndrome carrying a *PTPN11* mutation.

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Noonan syndrome (NS) is characterized by short stature, facial dysmorphisms and congenital heart defects. Mutations in *PTPN11* are the most common cause of NS. Patients with NS have a predisposition for developing leukemia and specific solid tumors. Exact data on the incidence of malignancies in NS are lacking. Consequently, the necessity of a surveillance program is unclear.

We performed a historical cohort study among 297 Dutch NS patients with a germline mutation in *PTPN11* (median age 12.7 years, range 0.6-94.8 years). The cancer histories were derived based on the referral forms for DNA diagnostics, by consulting PALGA, a nationwide registry of pathology in the Netherlands, and the Netherlands Cancer Registry. The reported numbers of cancer in the patients with NS were compared with the expected numbers based on population-based incidence rates.

Twelve patients with NS developed a malignancy, yielding a cumulative risk of developing cancer of 23.0% [95% confidence interval (CI), 7.6-38.4%] up to age 55, which represents a 3.5-fold [95% CI, 2.0-5.9] increased risk compared to the general population. Haematological malignancies occurred most frequently. Two rare malignancies were found not yet observed in NS, being malignant mastocytosis and malignant epithelioid angiosarcoma. We did not find a correlation between specific mutations in *PTPN11* and the occurrence of cancer.

This first large cohort study of cancer incidence in patients with NS and a mutation in *PTPN11*, shows an excess risk of cancer compared to the general population. Our data do not warrant a specific cancer surveillance program for patients with NS.

P02.143 Spectrum of anomalies in Polish patients with Noonan syndrome and N308D, N308S mutations in *PTPN11* gene

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Noonan syndrome (NS) is a congenital condition inherited in an autosomal dominant manner. The associated abnormalities include short stature, facial and skeletal dysmorphisms, cardiovascular and haematological defects and lymphatic dysplasias. Approximately 50% of NS cases are caused by mutations in the *PTPN11* gene encoding the Src homology protein-tyrosine phosphatase-2 (SHP-2) acting in the Ras-MAPK signaling pathway. The p.N308D amino acid substitution represents the most prevalent mutation (nearly 20%) in NS patients. We present Polish patients with Noonan syndrome and mutations p.N308D and p.N308S in *PTPN11* gene. The molecular analysis of *PTPN11* gene in a group of 58 unrelated patients with Noonan syndrome revealed the c.922A>G (p.N308D) mutation in five patients and

the c.923A>G (p.N308S) mutation in two patients. In total, mutations affecting the 308 amino acid position were identified in 12% of investigated patients. Three mutations were of maternal origin, one appeared *de novo*, in three cases parental DNA probes were not available for the analysis. Moreover, the mutations were revealed in other members of patients' families. All the patients presented clinical features typical for NS, although in various degree, e.g. short stature (height < 3 pc) was noted in five patients, chest deformity in all cases, bilateral undescended testes in all male cases, relative macrocephaly in three patients. Of note, the high frequency of congenital heart defects (PVS, ASD, VSD, PFO, hypertrophic cardiomyopathy) and learning difficulties (with normal / borderline IQ level) were observed.

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P02.144 Cardiac findings in 61 Noonan syndrome patients with proven mutations in genes of the RAS/MAPK signaling pathway.

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Noonan syndrome is an autosomal dominant disorder due to mutations in genes of the RAS/MAPK signaling pathway. The cardiac involvement is the one that poses more threatening outcomes.

We evaluated the cardiac findings in 43 patients presenting *PTPN11*, 13 with *SOS1* and 5 with *RAF1* gene mutations.

Cardiac abnormality was present in 53/61 patients (87%). The most frequent one was pulmonary stenosis (37/53 -70%), followed by hypertrophic cardiomyopathy (8/53 - 15%), atrioventricular septal defect (3/53 - 6%), isolated septal defects (2/53 - 4%), mitral valve prolapse (2/53 - 4%) and Ebstein anomaly (1/53 - 2%). In the group of patients with pulmonary stenosis, 20/37 (54%) required a surgical procedure and in 2/6 a balloon pulmonary valvuloplasty was not effective. In accordance to the literature, in patients presenting *RAF1* gene mutations, 4/5 presented hypertrophic cardiomyopathy and among the individuals with *PTPN11* gene mutations, 26/36 (72%) presented pulmonary stenosis. Ebstein anomaly (disclosed here in a patient with *SOS1* gene mutation) was rarely described in Noonan syndrome and its genetic mechanisms are still uncovered. Based on these data, 26 patients presenting this cardiac anomaly as an isolated form was screened for *SOS1* gene mutations, but no abnormalities in the coding region of this gene was found, suggesting that this gene is not a main one in the etiology of this particular cardiac abnormality.

Our data supports previous reports emphasizing that cardiac abnormalities in Noonan syndrome are particularly important, with high frequency of surgical procedures in patients presenting pulmonary stenosis. (FAPESP).

P02.145 Identification of co-occurring *SHOC2* and *PTPN11* mutations in a patient with Noonan syndrome

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Noonan Syndrome (NS) is an autosomal dominant disorder associated with short stature, congenital heart defect and facial dysmorphism, and a variable clinical expression. NS is a heterogeneous disorder caused by activating mutations in *PTPN11* (50%), or in *SOS1*, *RAF1*, *KRAS*, *BRAF*, *MEK1*, *MEK2*, *NRAS* and *SHOC2* was conducted revealing the co-occurrence of two previously identified mutations in the index patient. The mutation *SHOC2* c.4A>G; p.S2G represents a *de novo* mutation, whereas the mutation in *PTPN11* c.1226G>C; p.G409A is of maternal origin. The mother had no clinical evidence of NS but short stature, supporting that the *PTPN11* p.G409A mutation represents a mild par-

Here, we present a clinical and molecular characterization of a patient with NS phenotype and associated features including mild psychomotor developmental delay, hoarse voice, osteoporosis, loose anagen hair, gingival hyperplasia, spinal neuroblastoma and liver haemangioma. Mutation analysis of *PTPN11*, *SOS1*, *RAF1*, *KRAS*, *BRAF*, *MEK1*, *MEK2*, *NRAS* and *SHOC2* was conducted revealing the co-occurrence of two previously identified mutations in the index patient. The mutation *SHOC2* c.4A>G; p.S2G represents a *de novo* mutation, whereas the mutation in *PTPN11* c.1226G>C; p.G409A is of maternal origin. The mother had no clinical evidence of NS but short stature, supporting that the *PTPN11* p.G409A mutation represents a mild par-

tial mutation.

We propose that the atypical phenotype of the NS patient reported here is the result of an additive effect where the mild *PTPN11* mutation acts as a modifier. Interestingly, co-occurrence of RAS-MAPK mutations has previously been found in a few patients with variable NS or NFNS phenotypes. Taken together, the results suggest that the concurrence of mutations in the RAS-MAPK pathway may contribute to the clinical variability observed among NS patients.

P02.146 SOS1 gene mutations in Polish patients with Noonan syndrome

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Noonan syndrome (NS), an autosomal dominant disorder is characterized by congenital heart defects, short stature, facial dysmorphism, chest deformity, scoliosis, hearing loss, motor and neurocognitive delay, various coagulation defects, lymphatic dysplasias and tumorigenesis predisposition. Heterozygous gain of function mutations in various genes (*PTPN11*, *SOS1*, *RAF1*, *KRAS*) encoding proteins of the Ras-MAPK signalling pathway have been identified as the genetic basis of NS. Mutations of *SOS1*, the gene encoding a guanine nucleotide exchange factor for Ras, are the second major cause of Noonan syndrome after *PTPN11* mutations and account for 17-20% individuals. The aim of the study was to determine the *SOS1* gene mutation rate and profile in a cohort of well-characterized 43 unrelated Polish patients with Noonan syndrome lacking *PTPN11* mutations and to study the genotype-phenotype correlation. Molecular analysis of *SOS1* gene revealed six recurrent mutations (p.M269T, p.M269R, p.G434K, p.L550P p.R552K, p.R552G) in 8 affected individuals (18.6%). All mutations occurred *de novo* and are known or predicted to disrupt autoinhibition of *SOS1* RAS-EGF activity. The various congenital heart anomalies (pulmonary valve stenosis, ventricular septal defect, and mitral valve anomaly) and short stature were observed as being the most frequent. Retarded speech development was observed in one patient, while all cases have normal level of cognitive function. In comparison to the group of cases with *PTPN11* gene mutations, patients with *SOS1* gene mutations show higher frequency of ectodermal abnormalities (keratosis pilaris, wavy hair, sparse eyebrows, ulerythema ophryogenes). The study was supported by MNiSW Project PB 0056/B/P01/2008/35 and by CMHI project 190/08.

P02.147 De novo deletion of *NRXN1* in a girl with mild developmental delay and speech development disorder

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Mutations in the *NRXN1* gene have been associated with a wide range of neurodevelopmental and neuropsychiatric disorders, such as autism spectrum disorder, mental retardation and schizophrenia. Recently, dominant as well as recessive mutations in the *NRXN1* gene have been described to cause developmental delay of variable degrees. Here we report the phenotype of a 6 year old girl who was referred to our clinic because of discrete facial dysmorphisms, mild developmental delay with an emphasis on speech development problems. Molecular karyotyping was performed on the patient showing an approximately 462 kb loss of genomic material in 2p16.3 encompassing the 5' region and several exons of the *NRXN1* gene. Array-CGH analysis of the parents revealed no aberration indicating that the deletion in the patient has occurred *de novo*. The clinical implication of *NRXN1* deletions is not clear. The *NRXN1* gene locus is known to harbor a number of benign copy number variants (CNVs). High phenotypical variability and reduced penetrance make *NRXN1* deletions difficult to assess. It has been proposed that exon disrupting deletions in *NRXN1* are more likely to be pathogenic. Our case report supports this idea and broadens the spectrum of phenotypes.

P02.148 Investigation of "extreme" patients sheds light on new mechanisms of obesity

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Investigation of carefully-selected patients with extreme phenotypes may shed light on the mechanisms of common diseases. We aimed to identify genomic structural variants that could underlie the phenotypes of 34 dysmorphic children with obesity and mental retardation, with the hope of uncovering new loci that may be associated with common obesity. We have previously reported the discovery of causative deletions at chromosome 15q11-3 (the Prader-Willi syndrome region) and at chromosome 16p11.2. Also detected in this cohort were 19 further aberrations over 50kb in size and not overlapping known copy number variants, including: a ~1Mb duplicated region at chromosome Xq12; a ~500kb duplication at Xq13.2; a ~500kb duplication at 6p12.3; and a ~175kb deletion at 19p13.3. Characterisation of the latter deletion showed that it had arisen *de novo*, as it was not found in the patient's parents, and that one of the breakpoints lay within the *MAP2K2* gene, mutations in which cause cardiofaciocutaneous syndrome. The deletion includes *ZBTB7A*, a good candidate gene for the patient's obesity and cognitive phenotypes: this gene is involved in adipogenesis and mouse knockout models have revealed disruption of the Notch pathway, which is central for neuronal development. Similar analysis of the other candidate regions is ongoing, and it is possible that additional rare variants contributing to the genetics of common obesity may be identified, as for the 16p11.2 deletion which accounts for 0.7% of morbid obesity in the general population.

P02.149 Odontoonychodermal Displasia: A case report

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Odontoonychodermal Displasia (OD)(OMIM 257980) which is also called Fadhil Syndrome is an otosomal recessive disorder characterized by ectoderm anomalies including hyperhidrosis, hyperkeratosis palmaris, distrophy of nails, dry and sparse hair, facial erythema, geg-shaped incisors and malformed teeth. The aim of this case report is to present a patient with odontoonychodermal dysplasia with clinical and radiographical features. Our case is a 10-year-old boy was attended to clinic with a chief complaint of teeth crowding . He has sparse hair , dry skin , planter fissur on the feet . Clinical and radiographical examination revealed Angle Class I malocclusion , oligodontia , eruption cyst on right upper first molar teeth, and conical-shaped tooth. In accordance of this findings a diagnosis of odontoonychodermal dysplasia was made.

P02.150 A case report:Onycotrichodysplasia with mental retardation but without neutropenia

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A four years old boy who presented with abnormal hair was found to have sparse, short, dry, curly hair. His hair was never been cut. Hair microscopy concluded as trichoreksis nodosa. He has dysplastic nails. His nails of hand and foot finger is spoon-shaped. Eyebrow is sparse at medial and absent at lateral. Eyelash is absent at down eyelid. He has mild mental retardation (IQ:70). Cranial Magnetic Resonance Imaging and skeletal radiography showed normal findings. A diagnosis of onycotrichodysplasia with mental retardation was made. Onychotrichodysplasia is a rare autosomal recessive disorder. First case is shown by Cantu et al. (1975) Cantu et al. (1975) described a male infant with hypoplastic fingernails, trichorrhexis, chronic neutropenia, and psychomotor retardation. In contrast to cases described earlier, our patient has normal neutrophil .

P02.151 Osteogenesis Imperfecta subtypes - Importance to the clinic?

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Osteogenesis Imperfecta - OI - is a heterogeneous group of diseases characterized by susceptibility to bone fractures with variable severity and presumed or evidenced defects in collagen type I biosynthesis. The definition and classification of OI have been subject of debate, with recent addition to four new types (V-VIII) to the original Sillence classification. This has, indeed, generated confusing statements in the literature, and added complexity to the diagnosis.

The present work describes 10 cases of Portuguese children and adolescents with OI. There is no consensus on how to classify these cases among specialists. Should further diagnostic tests, including the analysis of bone histology and mutation screening in the genes *LEPRE1*, *CRTAP* and *PPIB*, which are not yet routinely performed, be pursued irrespective of the desire of prenatal diagnosis? How important is it to subclassify these patients? What will it add to the management and care of these patients? The aim of this poster is to generate debate and consensus.

P02.152 Case Report - Autosomal Recessive Infantile Malignant Osteopetrosis in India with novel mutation in TCIRG1 gene

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Autosomal recessive malignant osteopetrosis (OPTB, OMIM no. 259700) is a severe form of inherited osteosclerosis. The disease is rare and is found all over the world. We report the first case of OPTB proven by mutation analysis in India. A boy at the age of 4 months had neonatal seizure with hypoglycaemia and hypocalcaemia. On examination he was found to have mild hepatomegaly and moderate splenomegaly; mild anemia (11.4 gm%), thrombocytopenia (68,000/cumm), increased retic count (8%), increased alkaline phosphatase serum level (1883 IU/L). His X rays revealed evidence of osteopetrosis with dense cortex and obliteration of marrow. The patient is first born child on second degree consanguineous parents; there is no family history of similar illness. The DNA sequencing of the entire coding region and exon-intron junctions of TCIRG1 gene revealed the mutation c.1276C>T at the homozygous state in a patient. The mutation results in premature stop-codon formation (p.Arg426X) in osteoclastspecific a3 subunit of the vacuolar ATPase proton pump, in which various defects cause most cases of OPTB.

P02.153 Partial trisomy of the long arm of chromosome 6 in a family: clinical variability among affected members

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Partial duplication of the long arm of chromosome 6 is a rare cytogenetic abnormality identified in about 40 patients. It causes a recognizable syndrome characterized by: microcephaly, downward slanting palpebral fissures, telecanthus, micrognathia, carp shaped mouth, short neck and severe psychomotor retardation. The minimal critical region for the clinical phenotypic manifestations of the syndrome includes the 6q25-26 bands.

We describe a partial 6q trisomy in four related patients, as the result of an abnormal segregation of balanced maternal interchromosomal insertion. Probands facial dysmorphisms included: telecanthus, depressed nasal bridge, anteverted nostrils, short neck. One patient showed moderate mental retardation, another one only mild neurological impairment and interatrial septum defect. The remaining two patients had normal psychomotor development. The probands' karyotype confirmed by FISH and array-CGH analysis showed: "dup(6)(q22q23)". Karyotype analysis of balanced translocation carriers confirmed by FISH showed: 46 XX, ins(12;6)(q22;q22q23).

The patients showing 6q22-23 partial trisomy share the dysmorphisms of the so called "6q syndrome"; they didn't have severe mental retar-

dation, described in the syndrome. Our data could suggest that the 6q22-23 duplication cause a mild "6q syndrome".

P02.154 Nail Patella Syndrome: A Case Report

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An 11 year-old-boy was taken to the orthopedic clinic because of the pain on his right knee. Patellar hypoplasia was determined in the MR report at the right knee. Then the patient referred to the pediatry clinic for further investigation. In the physical examination the supination and extantion of the right elbow was restricted, nail of the first and second fingers were dysplastic and there was morphological defect on the both knee. X-ray imaging of the right elbow revealed that the medial chondyle of the humerus, the head of the proximal radius was hypoplastic and the lateral angulation was seen, also dislocation was found. Both patellar contours were irregular and hypoplastic at the X-ray imaging of the knee. Patella laterally dislocated. Bilateral distance of knee joints were laterally narrowed. The spina bifida was detected at the anteroposterior pelvic X-ray. The patient thought to be the Nail Patella Syndrome and referred to the ophthalmologist for the investigation of the glaucoma, microcornea and cataract. No pathology was found. There were no pathological findings at the other laboratory findings and physical examinations except of the urinary incontinence

P02.155 Identification of Pelizaeus-Merzbacher disease in a two-year-old boy

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A two year old boy was referred due to psychomotor developmental delay, nystagmus and hypotonia. He could not crawl, walk, nor sit. The nystagmus was first noticed when he was six weeks old. He had five words and no distinct dysmorphic features. He had an abnormal fat distribution on his thighs and pubic area. The family history was negative.

Investigations: SMA, Myotonic Dystrophy, Prader-Willi, CDG Ia and karyotype were normal. MRI scans showed thin corpus callosum and marked dysmyelination. ArrayCGH identified a 500kb duplication of Xq22.2, including the *PLP1* gene.

Background: Pelizaeus-Merzbacher (PMD) disease is a rare X-linked genetic disorder caused by deletions, duplications or point mutations in the *proteolipid protein 1 (PLP1)* gene. PLP1 is the major structural protein of the CNS myelin and mutations in the gene result in dysmyelination. Mutations in *PLP1* produce a wide spectrum of clinical phenotypes, from PMD to spastic paraparesia type 2. Boys are affected and their mothers are usually asymptomatic carriers. In common with most cases (50-70%) our patient had a duplication of *PLP1* as the causative mechanism and presented with classic PMD. Some patients have three or more copies of *PLP1*, which usually causes a more severe phenotype. Missense mutations often result in the connatal form, the most severe form of PMD. Clinical signs may be nystagmus, hypotonia, ataxia, titubation, cognitive impairment and spastic quadriplegia beginning in the first five years. In severe cases perinatal stridor and seizures may develop.

P02.156 Genetic variants in DRD2 gene and therapeutic response to antipsychotic treatment in schizophrenia patients from Volga-Ural region of Russia: pharmacogenetic study

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Antipsychotic drugs exert both therapeutic and adverse effects through dopamine D2 receptor (DRD2) antagonism. Genetic variants of this receptor may be responsible for individual variations in antipsychotics response and may therefore be useful in predicting response. In this study we evaluated the role of two polymorphic loci: TaqI A and NcoI of DRD2 gene in 120 drug-naïve patients (Russians and Tatars) with first-episode schizophrenia, from Volga-Ural region of Russia who treated with typical antipsychotics for 45 days. Improvement and response was assessed by using the Positive and Negative Syndrome Scale (PANSS) on the day of admission and subsequently after 21 and

45 days following the treatment. Association tests between genotypes and percentage improvement in total PANSS score, as well as Positive and Negative subscale scores were performed using analysis of variance (ANOVA). Taql A polymorphic locus of DRD2 gene was associated with Positive symptom response to treatment. Patients with DRD2*A1/A1 genotype showed substantial improvement as regards Positive symptom response (F4.867; df 2; P=0.009) compared with patients who carried DRD2*A1/A2 and DRD2*A2/A2 genotypes. No association was observed between Nco polymorphic locus of DRD2 gene and changes in PANSS scores. The results suggest that Taql A polymorphic locus of DRD2 gene may be a useful predictor of reduction in positive symptoms in schizophrenia patients treated with typical antipsychotics.

P02.157 A family with Pierre Robin syndrome caused by a microdeletion in the long arm of chromosome 17 (17q24.3)

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Pierre Robin syndrome or sequence (OMIM 261800) is defined by micrognathia, cleft palate and glossoptosis, resulting in upper airway obstruction, neonatal distress and feeding problems that can be life-threatening. This sequence appears also as a feature of campomelic dysplasia, an autosomal dominant short limb dwarfism, caused by mutations in the SOX9 gene.

We report on a male child, his mother and grandmother. The child presented at birth with an important retrorocketognathia, a posterior U-shaped cleft palate, a left palmar crease, and normal birth measurements. His mother also had a Pierre Robin syndrome (with cleft palate), diagnosed at birth, that was followed by multiple hospitalisations and surgical interventions. The maternal grandmother also had a history of cleft palate and surgery. Further family history revealed that 3 brothers and 1 sister of the maternal grandmother died in the neonatal period because of "problems of the face". The child, mother nor grandmother have other associated anomalies. Chromosome analyses and FISH 22q11.2 were normal.

Affymetrix SNP chip analyses (250K NspI) revealed a small deletion of approximately 249 kb localized in 17q24.3 in the child, the mother and the maternal grandmother. There is no known gene or polymorphism in this region but it is situated at 1.3Mb of the SOX9 gene. This microdeletion is thus very probably causative for the Pierre Robin syndrome in this autosomal dominant family. A possible role of SOX9 in the etiology of Pierre Robin sequence seems to be confirmed by recent findings in the literature.

P02.158 4G/5G Polymorphism of PAI-1 Gene is Associated with Multiple Organ Dysfunction and Septic Shock in Pneumonia Induced Severe Sepsis

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Activation of inflammation and coagulation are closely related and mutually interdependent in sepsis. The acute-phase protein, plasminogen activator inhibitor-1 (PAI-1) is a key element in the inhibition of fibrinolysis. Elevated levels of PAI-1 have been related to worse outcome in pneumonia. We aimed to evaluate the effect of functionally relevant 4G/5G polymorphism of PAI-1 gene in pneumonia-induced sepsis.

We enrolled 207 Caucasian patients with severe sepsis due to pneumonia admitted to an intensive care unit (ICU). Patients were followed up until ICU discharge or death. Clinical data were collected prospectively and the PAI-1 4G/5G polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism technique. Patients were stratified according to occurrence of multiple organ dysfunction syndrome, septic shock or death.

Our results showed that carriers of the PAI-1 4G/4G and 4G/5G genotypes have 2.74-fold higher risk for multiple organ dysfunction syn-

drome (p=0.005) and 2.57-fold higher risk for septic shock (p=0.015) than 5G/5G carriers. The multivariate logistic regression analysis adjusted for independent predictors, such as age, nosocomial pneumonia and positive blood culture also supported that carriers of the 4G allele have higher prevalence of multiple organ dysfunction syndrome (p=0.009) and septic shock (p=0.024) than patients bearing the 5G/5G. However, genotype and allele analyses have not shown any significant difference regarding mortality in models non-adjusted or adjusted with APACHE II.

Carriers of the 4G allele of PAI-1 polymorphism have higher risk for multiple organ dysfunction syndrome and septic shock in Caucasian patients with severe sepsis due to pneumonia.

P02.159 Quantitative SNRPN methylation analysis in neonates with central hypotonia

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The hypotonic infant is an issue in pediatric neurology, this condition can be due to many different pathological processes in brain or any structure in the motor unit. In a central hypotonic baby approach is important discard syndromic causes, being the most frequent, Prader Willi Syndrome (PWS) caused by the loss of expression of the paternal allele in a group of imprinted genes located in a 2 Mb domain within 15q11-q13. PWS is characterized by severe prenatal and postnatal hypotonia. SNURF-SNRPN gene methylation analysis in the critical region detect 99% of the cases but does not provide information about the etiology of the syndrome, FISH analysis the choice tool to detect microdeletions. Real time PCR analysis could make the diagnosis, verify deletions and detect mosaicism in one reaction. In infants the diagnosis is difficult, clinical approach alone is not enough to differentiate this condition from others. It has been proposed that around 40% of the hypotonic patients have PWS but an accurate percentage has not been established. SNRNP-Quantification of methylated alleles (QAMA) in 24 central hypotonic infants showed in 5/13 patients (41.5%) with PWS. QAMA permits calculate gene dosage with the comparative C_T method and detect those cases with microdeletion.

P02.160 Prader - Willi Syndrome - Classic Disorder, New Features

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Prader Willi Syndrome (PWS) is a relatively common disorder (1/15,000 newborns) due to abnormalities in the 15q11.2-q13 region (microdeletion, uniparental disomy, methylation defect, deletion).

Major manifestations include hypotonia with poor suck and poor weight gain in infancy, early childhood-onset hyperphagia and obesity, characteristic appearance, hypogonadism, growth hormone insufficiency causing short stature, mild mental retardation and characteristic behaviour. Diagnostic is based on a clinical score (Cassidy 2005). Selected cases follow investigations (methylation test, FISH, karyotype). We present the clinical study of 50 cases with PWS (diagnosis confirmed by genetic tests), in order to illustrate and discuss particular clinical features. Some of them are relatively frequent in our patients (e.g. multiple allergies, tall/ normal stature in childhood, long ears, early onset puberty, particular behavior, normal appetite etc) and should be considered when evaluating a patient. Our results are comprehensively illustrated, discussed and compared with literature data. In conclusion, we present a clinical study of PWS that illustrates particular features relatively frequently found in our patients.

P02.161 New perspectives on Prader-Willi Syndrome: report of 2 patients and review of the literature

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Prader-Willi syndrome (PWS; MIM #176270), a rare genetic disorder, is an example of a genetic condition involving genomic imprinting and

the first shown to be caused by uniparental disomy. It can occur by three main mechanisms, which lead to absence of expression of paternally inherited genes in the 15q11.2-q13 region: paternal microdeletion (70%), maternal uniparental disomy (25%), and mutation or other abnormality in the imprinting process (2% to 5%). Although caused by the disturbed expression of genes from the imprinted region of 15q11-q13, the specific contributions of individual genes remain unknown. PWS is a neurodevelopment disorder, with an estimated prevalence of 1 in 15.000, with a complex phenotype that changes with age. The most consistent major manifestations include neonatal hypotonia, short stature, global developmental delay and mental deficiency, behavioral and sometimes psychiatric abnormalities, early childhood-onset hyperphagia and obesity, hypothalamic hypogonadism, and characteristic appearance.

Diagnosis of PWS on a clinical basis only is difficult in newborns and young infants; thus cytogenetic approaches such as fluorescent *in situ* hybridization combined with molecular tests like DNA methylation test and UPD studies are essential. Selection of suitable patients based on clinical criteria according to age, enhances diagnostic yield.

We describe the clinical, cytogenetic and molecular characterization of 2 patients with PWS and discuss the new perspectives on therapeutic and educational programmes, rehabilitation issues and the potential role of the genes within the deleted region in the pathogenesis of these various phenotypic abnormalities.

P02.162 Prevalence of Prader-Willi and FRAXA syndromes among patients with mental retardation

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One of the main tasks of medical genetic consulting is timely and correct diagnostics of diseases. However, there are several groups of pathologies including mental retardation, which are characterized by broad clinical polymorphism and genetic heterogeneity. The aim of present investigation was assessment of Prader-Willi and FRAXA syndromes prevalence among children with intellectual disorders in Novosibirsk region. Among 433 patients clinical diagnosis of Prader-Willi syndrome (PWS) was suspected for 22 children (5.1%). Results of molecular genetic analyses were positive in 10 cases (45%). One family with two affected sibs and unusual mechanism of inheritance was described. One child had microdeletion of 15q11-q13 on paternal chromosome, whereas the other UPD(15)mat. FRAXA syndrome was suspected for 25 patients (5.8%) on the basis of clinical examination. Combined cytogenetic and molecular genetic tests were positive for 11 children only (44%). Our experience indicates that about a half of the patients with clinical features of frequent mental retardation syndromes and negative testing results require more detailed molecular genetic analysis.

P02.163 Achondroplasia - case report

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Introduction: Achondroplasia, a non lethal form of chondrodysplasia, is the most common form of short-limb dwarfism. Positive diagnosis is relatively easily established based on salient phenotypic features that include: disproportionate short stature, megacephaly, a prominent forehead (frontal bossing), middle face hypoplasia, rhizomelic shortening of the arms and legs, a normal trunk length, thoraco lumbar gibbus (lumbar kyphosis) genu varum, and a trident hand configuration.

Materials and method: Premature newborn - cytogenetic, imaging and clinical examination.

Results: L.M new born, aged 4 days was admitted to our clinic presenting a plurimalformativ syndrome. The patient comes from clinically healthy parents, gestational age was 37 weeks, birth weight was 2260g, the length = 35 cm and Apgar index 1 to 5 minutes. The disease was diagnosed in the 6th month of pregnancy by fetal ultrasonography.

Positive diagnosis was established based on salient phenotypic features: middle face hypoplasia, a prominent forehead (frontal bossing), moderate exophthalmia, posterior palatoschizis, shortening of the arms and legs (the arms = 13 cm), short global stature, sitting down

stature = 30 cm and varus equine.

Diagnosis was also supported by other investigations:

Radiographic findings: shortened long bones of the limbs with epiphysis osteocondritis

Genetic study: without changes in karyotype

Evolution during neonatal period was difficult with slow growth in weight, due to infections, (especially respiratory) and eating difficulties.

Conclusion: Achondroplasia is a rare disease in current medical practice. Diagnosis is established easily, both antenatal and postnatal period, based on clinical data, imaging and cytogenetic testing.

P02.164 Mitochondrial DNA analysis in primary congenital glaucoma

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Purpose: To identify mitochondrial DNA (mtDNA) nucleotide changes in primary congenital glaucoma (PCG). Methods: The entire coding region of the mitochondrial genome was amplified by polymerase chain reaction from 35 patients and 40 controls. Whole mtDNA genome except D-loop was sequenced. All sequences were analyzed against mitochondrial reference sequence NC_012920. Results: MtDNA sequencing revealed a total of 132 and 58 nucleotide variations in PCG and controls respectively. Thirty one novel mtDNA variations were detected in PCG. Of 132 nucleotide variations, 41 (31.06%) were non-synonymous and 83 (62.87%) were synonymous changes, and 8 were in RNA genes. Highest numbers of nucleotide variations were recorded in complex I followed by complex IV then complex V. Two non-synonymous changes (p.W239C in ND2 and p.A20T in ATPase6) were present both in cases and controls. Discussion: Mitochondrial function can be affected by mutations in mitochondrial and nuclear DNA. MtDNA variations result in reduced mitochondrial respiration and elevated reactive oxygen species (ROS) production. Reduced mitochondrial respiration may lead to trabecular dysgenesis which is a characteristic feature of PCG. OS affects both TM and retinal ganglion cells (RGCs) and is involved in the neuronal cell death affecting the optic nerve. Conclusion: Mitochondrial DNA variations adversely affect respiratory chain and impair OXPHOS pathway and result in reduced mitochondrial respiration and elevated ROS production. This leads to oxidative injury to TM and RGCs. Thus, early identification of mitochondrial DNA variations and prompt antioxidant administration may delay oxidative stress induced injury to TM and RGCs and, therefore, improve visual prognosis.

P02.165 Salt Loosing Form of Cystic Fibrosis (Pseudo-Bartter Syndrome): A Case Report

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11 months girl patient admitted to our emergency clinic with vomiting, cough and diarrhea complaints. She had clinical picture of PBS and her sweat test results was suspicious and to confirm CF diagnosis we apply to supporting findings. Patient diagnosed with salt loosing form of CF (Pseudo Bartter's Syndrome). Treatment with appropriate antibiotics, intravenous fluid and electrolyte replacement biochemical finding and general condition of patient improved. She discharged from a hospital with an oral salt and potassium support.

This case presented on purpose to underline the distinctive diagnosis of PBS related with CF infants which are applied with an electrolyte imbalance and metabolic alkalosis particularly in a hot climate.

P02.166 A new storage disorder resembling Morquio syndrome in two sibs

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We report two brothers born to unrelated French Caribbean parents presenting with an unclassifiable storage disorder. Pregnancy and delivery were uneventful. They have short stature (below - 4SD) with

short trunk, barrel chest, micromelia with rhizomelic shortening, severe kyphoscoliosis, pectus carinatum, ulnar deviation of the hands, short hands and feet with metatarsus adductus, excessive joint laxity of the small joints and umbilical hernia. Mild developmental delay was present in both. There was no hepatomegaly or splenomegaly, nor dysmorphism. Skeletal X rays demonstrated generalized platyspondyly with a tongue-like deformity of the anterior part of the vertebral bodies (reminiscent of Morquio syndrome), and hypoplasia of the odontoid. Generalized epiphyseal dysplasia and abnormally shaped metaphyses were also present. Ophthalmologic examination was normal. Spine deformity required surgical correction in one of the patient at age 4. Despite similarities with mucopolysaccharidosis IV, lysosomal enzymes assays including alpha galactose-6-sulfatase and beta-galactosidase were normal on lymphocytes. Traces of urinary glycoaminoglycans were found in one of the two brothers. They were no vacuolized lymphocytes, but abnormal coarse inclusions were present in the eosinophils. The azurophiles granulations of the polymorphonuclears were also abnormal. Those morphological anomalies are indicative of a lysosomal dysfunction. Skin biopsy was normal. Ultrastructural examination of the cartilage is pending.

We hypothesize that these two boys have an undescribed lysosomal storage disorder of unknown origin that share clinical and radiological features with Morquio disease.

P02.167 An atypical case of pseudoxanthoma elasticum with abdominal cutis laxa: evidence for a clinical disease spectrum

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Introduction. Pseudoxanthoma elasticum (PXE), featuring papular skin lesions, a retinopathy and cardiovascular complications, results from elastic fibre calcification and fragmentation due to ABCC6 mutations. The PXE-like syndrome (PXL), caused by GGCX mutations, features generalized cutis laxa, mild retinopathy and a clotting deficiency. The GGCX carboxylase activates vitamin K (VK)-dependent calcification inhibitors {matrix gla protein (MGP), osteocalcin (OC)}. We present a patient with a clinical and histochemical overlap phenotype between PXE and PXL.

Methods & Results. The proband presented typical PXE features - yellowish papules in the neck and severe retinopathy - together with marked abdominal cutis laxa, as in PXL. Dermal ultrastructural evaluation revealed mineralization in the periphery of elastic fibres, typical for PXL. Immunohistochemistry showed marked staining for uncarboxylated (uc) MGP and ucOC, seen in PXE and PXL, though not confined to the middermis - as in PXE - but affecting the whole dermis as in PXL. Measuring circulating levels of carboxylated (c) and ucMGP and OC revealed elevated ucOC/cOC ratios, as in PXL (normal in PXE), but normal ucMGP/cMGP ratios, as in PXE (elevated in PXL). Molecular analysis unveiled two known compound heterozygous ABCC6 mutations, while GGCX harboured a gain-of-function polymorphism. Circulating VK levels were severely decreased, possibly neutralising the effect of the latter.

Conclusion. This phenotype, reminiscent of PXE and PXL, suggests that PXL may represent a spectrum of ectopic calcification disorders who are clinically and pathogenetically related to PXE. The low VK serum levels suggest a pathophysiological role for a deficient VK status in these disorders.

P02.168 Ramon Syndrome in a 38-year-old male

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Ramon Syndrome (RS) was initially described by Ramon et al. (Oral Surg. 24:436-48, 1967). Since that time there have been few patients reported in the literature with this rare, presumed autosomal recessive syndrome. Described patients were mostly children. We report a new case of RS, a rare disorder, characterized primarily by gingival overgrowth, congenital generalized hypertrichosis, mental retardation and epilepsy.

Our proband was a Caucasian, 38-year-old male. Third son of non-

consanguineous parents and his older brothers of whom were unaffected. He was born full-term by normal vaginal delivery and his birth weight was 2kg. Developmental milestones were delayed. Reportedly, he attended regular school up to 9th grade. Our patient had mental deficiency, epilepsy, gingival fibromatosis, cherubism due to the fibrous dysplasia of the maxillae, hypertrichosis and pigmentary changes in the retina. Chromosomal study of peripheral blood lymphocytes confirmed the 46, XY karyotype.

Ocular features were not described in the original report on this syndrome. Recent reports of other families with R.S. describe pigmentary retinopathy and optic disc pallor. It may be concluded that ocular abnormalities are another feature of Ramon syndrome and are developing later.

P02.169 A novel mild phenotype associated with FOXG1 gene

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We recently identified FOXG1 gene as responsible for the congenital variant of Rett syndrome. This is the severe variant of the syndrome in which the normal perinatal period is very short or absent. After the first two Italian patients more than 20 patients have been further described. These patients represent a quite homogenous group showing mild postnatal growth deficiency, severe postnatal microcephaly, severe mental retardation with absent language development, midline stereotypes and jerky movements, poor eyecontact, irritability in infancy and gastroesophageal reflux. In this form, patients can neither speak nor walk and have severe dyspraxia. We present here a 12.5 year-old female with postnatal microcephaly (48 cm, 7.5 y), ataxic gait, hyperactivity, seizures, stereotypes, protruding tongue, prognathism, midface hypoplasia resembling 9qter microdeletion syndrome, normal karyotype and normal EHMT1 gene. Although she was able to walk, listening music, and manipulate objects (with moderate apraxia) we decided to test FOXG1 gene. A de novo early truncating mutation (c136C>T; p.Q46X) has been identified in this gene. To our knowledge this is the first report with a mild phenotype associated with FOXG1 mutation. This discovery paves the way to the possibility that also FOXG1 may be associated with mild variants, as for MECP2 gene in Zappella variant.

P02.170 A systematic metabolic approach to the evaluation of nutrition in Rett syndrome according to the cardiorespiratory phenotype in Dutch Rett girls.

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Background: Despite their good appetite, many females with Rett syndrome (RTT) meet the criteria for moderate to severe malnutrition. Although feeding difficulties may play a part in this, other constitutional factors as altered metabolic processes are suspected. Irregular breathing is a common clinical feature, which leads to chronic respiratory alkalosis or acidosis. The aim of this study was to examine the influence of breathing irregularities on metabolic processes, as a possible cause of impaired nutritional status.

Methods: The study population consisted of a well-defined group of thirteen Dutch RTT girls with complete clinical, molecular and neurophysiological work-up. A complete nutrition assessment and measurement of body composition was carried out. Blood and urine samples for biochemical screening of metabolites from multiple pathways were collected.

Results: Six RTT girls had a significantly elevated creatine concentration in plasma and creatine/creatinine ratio in urine. Five girls were forceful breathers, one girl had an undetermined cardiorespiratory phenotype. A significantly elevated creatine/creatinine ratio in urine only was seen in one girl, she was a feeble breather.

Conclusion: Chronic respiratory alkalosis may alter the creatine metabolism in females with RTT. Furthermore, MeCP2 deficiency may cause epigenetic aberrations affecting the expression of the creatine-transporter gene, which is located at Xq28. Further studies concerning the nutritional and cardiorespiratory requirements of RTT girls are important in order for them to receive appropriate and effective treatment.

P02.171 Ophthalmologic findings in one case of Rieger syndrome

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Rieger syndrome or mesodermal dysgenesis of the iris and retina (MIM180500, 4q25-q27) is a consequence of abnormal cleavage of the anterior chamber. This results in iris hypoplasia and strands running from the iris to the posterior surface of the cornea (synachiae). Posterior embriotoxon is usually present and glaucoma may be a complication. Here we report on 14 years old girl suffered from Rieger syndrome. Her parents deny consanguinity. She is under our observation during 5 years. Proband's phenotype included short proportionate stature, shield-shaped chest, arachnodactyl, oligodontia, peg-shaped teeth. Extensive ophthalmologic studies showed bilateral inborn glaucoma and pronounced mesodermal dysgenesis of the iris and retina, posterior embriotoxon, microcornea (d~8mm vs. 11.5-12mm). In the angle of the anterior chamber residual mesodermal tissue was shown with biomicroscopy. Congenital stromal prelimbal cloudy cornea was found here. In addition proband's phenotype included the irregular oval pupils displaced towards nasal area and turned vis-à-vis. There were two false congenital pupils (the left eye). Using gonioscopy the angles of the anterior chambers were found to be closed but intraocular pressure was below 22 mmHg without hypotensive medication. Refractive myopia was revealed. Her mental intellectual faculties were excellent. Trophic medication and optic correction were applied.

P02.172 Autistic features and unusual behavior in a girl with ring chromosome 11: clinical, psychological and molecular cytogenetic characterization

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We report an 8 year-old Caucasian girl with a ring 11 chromosome presenting with microcephaly, short stature, intellectual disabilities, spastic-ataxic syndrome and autistic features. The majority of phenotypic features resemble those previously reported for r(11), whereas spastic-ataxic syndrome and autistic features are not. The proposita is a girl delivered at term to non-consanguineous parents. She has severe growth and psychomotor retardation, bicuspid aortic valve, congenital strabismus, hipoplastic thymus gland, pancreas dysfunction, "cafe-au-lait" pigmentary skin changes, partial ptosis, abnormal ear shape, high-arched palate, broad nasal bridge, shortening of III metatarsal bone. MRI has showed slight frontal lobe atrophy, hydrocephalus and myelination deficit. She has inconsistent eye contact, increased anxiety/fears and ritualistic, stereotyped, self-injurious behavior, and is nervous, depressive and tearfulness. Total CARS score is 36 (moderate autism). Repetitive Behavior Scale-Revised score is 37 (high scores for self-injurious, restricted and ritualistic/sameness behavior; median scores for stereotyped behavior; low score for compulsive behavior). Speech difficulties refer to rhinolalia and poor vocabulary. The girl has good concentration span and long-term verbal memory, being extremely good in gestalt recognition with occasional difficulties of common things recognition. Molecular cytogenetic characterization was performed by FISH with site-specific DNA probes for subtelomeric and interstitial chromosome 11 regions as well as by whole-genome BAC array 1Mb CGH. We found ring chromosome to be associated with loss of 11q24.1->11qter (121,411,392-134,916,587 or ~13.5Mb) without chromosomal DNA loss within 11pter. To the best of our knowledge, this is the first case of r(11) characterized by array CGH.

P02.173 Sensenbrenner syndrome (Cranioectodermal Dysplasia): A candidate human ciliopathy. Presentation of new cases, review of the literature and possible mechanisms.

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Cranioectodermal dysplasia (CED, Sensenbrenner syndrome, OMIM 218220) is a rare syndrome characterized by dolicocephaly/sagittal craniostenosis and ectodermal anomalies. The majority of individuals have short stature and normal intelligence although developmental delay has been reported. CED is presumably autosomal recessive based on sibling recurrence, and parental consanguinity. Several CED patients have developed tubulo-interstitial renal disease, hepatic fibrosis and retinitis pigmentosa supporting that CED is a congenital hepatofibrocystic syndrome (CHFS). The majority of CHFS are now known to be members of the growing class of human ciliopathies. Several of the early cases of CED were reported in the literature multiple times which was not appreciated in some of the previous published reviews of this condition.

Herein we report our experience with several new cases of CED. This includes a child born to consanguineous Tatarian parents with confirmed *situs inversus*- a prototypic ciliopathy feature. We have also seen 3 Hutterite children with CED features and developmental delay, and we have mapped their disorder to the short arm of chromosome 17. The nearly 400 genes in this region are currently being prioritized for sequencing based on published ciliary databases-an update will be provided. We will also review all the published CED cases and the core phenotype. If CED is indeed a ciliopathy a number of predictions can be made prior to the first gene being identified- genetic heterogeneity can be anticipated, patients will be seen with CED and features overlapping other known ciliopathies, and that alleles at other ciliary loci will modify phenotypic expression.

P02.174 SHORT syndrome: autosomal dominant transmission and prenatal diagnosis

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The SHORT syndrome was first described by Gorlin and Sensenbrenner in 1975 where the acronym stands for: S stature, H hyperextensible joints and/or hernia, O ocular depression, R Rieger anomaly (iris stroma hypoplasia, posterior embriotoxon and iridocorneal synechia), T teething delay. It has now been reported in more than 24 patients of the two sexes. A partial lipodystrophy of the thorax and the face gives them a progeroid facial appearance. Intellect is generally normal. Some inconstant features can be present as hearing loss and renal anomalies. Adults present a high risk of mellitus diabetes with insulin resistance. The inheritance is autosomal dominant and until now, the etiopathogeny remains unknown.

In 2001, we reported the observation of a 27-year-old affected woman with absence of familial history. A first pregnancy was complicated by severe diabetes requiring high doses of insulin allowing the birth of an unaffected girl born prematurely at 29 weeks. At the age of 8, she is in good health. The watch of a second pregnancy of the 33years-old index case confirmed the event of gestational diabetes. Ultrasound examination shows a craniofacial fetal microsomia with dysmorphic features at 27 weeks of gestation. This confirmed the autosomal dominant inheritance from mother to the fetus. The pregnancy was terminated. Even if features are common with Rieger or progeroid syndromes, no mutations in PITX2 or lamin A/C genes was detected. Array-CGH was normal.

P02.175 Small duplication of the GPC3 gene in a Spanish family as a newly recognized cause of Simpson-Golabi-Behmel syndrome.

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Simpson-Golabi-Behmel syndrome (SGBS) is an X-linked recessive overgrowth disorder characterized by prenatal onset of overgrowth,

characteristic facies, frequently mild to severe mental retardation and different associated congenital anomalies. Up to date only mutations or deletions of the glycan 3 (*GPC3*) gene have been reported associated to this syndrome.

Here we describe a three generation Spanish family with a clinically recognizable SGBS. Subsequently to negative mutational screening of *GPC3* gene in the proband, we decided to perform array-CGH analysis (180K Agilent array), and identified a small duplication of about 35 kb within *GPC3* gene whereas no other significant copy number variants were recognized in the rest of the genome. The rearrangement, was checked using a SALSA MLPA KIT P154 SGBS-*GPC3*-4 (MRC-Holland) assay which confirmed the duplication of exon 6 leading to disruption of the *GPC3* gene. Further analysis in the pedigree demonstrated that the rearrangement co-segregated with the disease. A total of eleven family members have been molecularly investigated. Two affected males presented basically with neonatal hypoglycaemia (in one), coarse face, macroglossia, overgrowth, normal or mild learning difficulties, and renal anomalies. The clinical phenotype in the four female carriers varies from absolute normal to mild clinical manifestations.

To our knowledge this is the first case of SGBS encompassing duplication of the *GPC3* gene reported so far. As already demonstrated for other genes, the molecular screening of the *GPC3* gene should always include a gene dosage analysis in order to exclude small deletions / duplications.

P02.176 A De novo Deletion of the *SMC1A* gene at Xp11.2 in a female associated with cleft lip and palate, overlapping fingers, refractory seizures and hemivertebrae

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The *SMC1A* (OMIM 300040) gene located at Xp11.2 is reported to escape X inactivation and is associated with a variant of Cornelia de Lange (CdLS). Previous research suggested that mechanistically the *SMC1A*-related CdLS is due to the mutant proteins that maintain a residual function in males and cause a dominant negative effect in females. We report a case of a *de novo* deletion at Xp11.2 which included a partial deletion of exons 19-25 in *SMC1A*. The proband is a term female born to non-consanguineous healthy parents. Her birth parameters and APGAR scores were normal. On examination, she had hypotonia, left cleft lip and cleft of the anterior part of the secondary palate, bilateral camptodactyly and overlapping of the 2nd and 3rd digits. She also had a small VSD and hemivertebrae at C4-C5 and T9-T11. Brain MRI showed mild increased T2 signal in the white matter of the frontal and parietal lobes and suggestion of volume loss. At 2 months of age she developed intractable seizures with episodes of apnea, desaturation and bradycardia. Her karyotype was 46,XX and microarray analysis also showed a maternally inherited duplication at 15q11.2 which did not include the *SNRPN* locus. To our knowledge, our case is the largest known deletion in *SMC1A*. Analysis of *SMC1A* expression is undertaken in the patient's lymphoblast line. If this shows lack of the mutant proteins, it will question the validity of the theory that in females, the condition is the result of a dominant negative effect (Liu et al., 2009).

P02.177 Hypogonadotropic hypogonadism due to mosaic SOX2 mutation, and anophthalmia/microphthalmia in two offspring born after assisted reproductive treatment

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Heterozygous mutations in the transcription factor SOX2 are the commonest single-gene cause of anophthalmia/microphthalmia (A/M). In some patients with eye anomalies and SOX2 mutations, hypogonadotropic hypogonadism (HH) has been reported. Deficiency of luteinizing hormone and follicle-stimulating hormone secretion often necessitates the use of assisted reproductive technology to assist fertility in individuals with HH.

We report two children with anophthalmia/microphthalmia who were born following infertility treatment of their mother who had HH. A novel heterozygous frameshift mutation in the SOX2 transactivation domain

was found in both children on sequence analysis. Their mother, who had isolated HH with no eye or other anomalies, was found to harbour the same SOX2 mutation at mosaic levels in a peripheral blood sample. Although most SOX2 mutations arise *de novo*, the recurrence in this family as a result of mosaicism in the mother highlights the importance of clinical and molecular evaluation of parents of children with anophthalmia due to a SOX2 mutation to enable accurate recurrence risk counselling.

This is the first report of HH in the absence of eye involvement in a SOX2 mutation patient. The SOX2 mutation was present in mosaic form in a peripheral blood sample and it was detectable using routine sequencing methods. Most SOX2 mutations have been ascertained through the study of patients with ocular abnormalities, and it is not known what proportion of patients with HH may harbour SOX2 mutations. This report has important implications for the evaluation of patients with isolated HH, particularly in the setting of infertility treatment.

P02.178 Stickler Syndrome and mental retardation in a patient with a *de novo* 12q13.11 microdeletion

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Proximal 12q deletions are rare events. To date only 2 cases have been reported with a 12q13 partially overlapping deletion [Gallego et al., 2000 (Case1); Tonoki et al., 1998 (Case2)]. We describe an 11-year-old girl with mental retardation, microcephaly, cleft palate and severe bilateral myopia.

Array-CGH analysis (Agilent 244K) revealed a *de novo* interstitial 12q13.11 deletion of 1.3 Mb. The deleted region encompasses 16 genes including *COL2A1* and *AMIGO2*. The former gene is known to cause Stickler Syndrome type I 'OMIM 108300' when haploinsufficient, while the latter is specifically expressed on fiber tracts of neuronal tissues and involved in their formation [Kuja-Panula et al., 2003]. We hypothesize that *AMIGO2* haploinsufficiency is responsible for the mental handicap of our patient, providing further arguments for the role of *AMIGO2* in mental development. Our findings support the hypothesis that the haploinsufficiency of *AMIGO2* is probably responsible for the mental handicap of our patient. Characterization of additional patients with intragenic mutations of this gene will help advance our understanding of its involvement in mental retardation.

P02.179 Stuve- Wiedemann: a newly confirmed case in a Chilean infant

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Introduction: Stuve- Wiedemann syndrome (STWS) is a rare autosomal recessive disorder characterized by a congenital bone dysplasia and autonomous dysregulation, frequently associated with early death. It was described by Stuve and Wiedemann in 1971. In 2004 Dagoneau et al. identified mutations in the leukaemia inhibitory factor receptor (*LIFR*) gene. We describe a Chilean infant whose diagnosis and confirmation was done with the cooperation of the Skeldys network.

Case Report: first daughter of consanguineous parents. Prenatal ultrasound showed long bones <p5. BW: 3140g, BL: 46cm, CC:33.3cm. She developed severe respiratory distress at birth requiring mechanical ventilation for 10 days.

Physical examination showed a normocephalic newborn, with rhizomelic shortening of the limbs, slightly bowed legs. She had a round face with low nasal bridge, high palate, short neck, and overlapping fingers. Intraoral reflexes were diminished, with absent corneal reflexes, and normal patellar reflexes. She was unable to swallow requiring a gastrostomy. She has presented frequent hyperthermic episodes accompanied by dehydration, sweating, respiratory distress and poor

perfusion. Infectious screening have been repeatedly negative. Now she is 7m old and despite several hospitalizations, growth development is adequate to her age.

Skeletal survey showed mild osteopenia, irregular metaphysis, shortening and bowing of femur and tibia. Molecular study confirmed a *LIFR* mutation present at the homozygote state.

We stress the importance of sharing the history and radiological studies of patients with a suspected though infrequent skeletal dysplasia in the skeldys group. This permitted to achieve a prompt and confirmed diagnosis in this case.

P02.180 RARE SUBMICROSCOPIC REARRANGEMENTS

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Submicroscopic rearrangements involving chromosome ends are responsible for the unexplained mental retardation and multiple congenital anomalies observed in some patients. These results provide a new and useful tool to inform families and for genetic counselling. We present three families with a cryptic reciprocal translocation, detected using subtelomere specific FISH probes, where proband inherited an unbalanced segregant from the mother.

Case 1. A 9-years-old girl, affected of severe mental retardation, dysmorphic face and malocclusion of teeth, strabismus, seizures and joint laxity. MR show hypoplasia of corpus callosum, periventricular heterotopia and white matter alteration. Patient has a monosomy 6qtel and a trisomy 15qtel inherited from her mother. A healthy brother also has the same mother's balanced translocation.

Case 2. A 5-year-old boy with severe mental retardation, dysmorphic face and velopatinal insufficiency, hypospadias and seizures. Patient has a monosomy 17qtel and trisomy 19qtel inherited from his mother.

Case 3. A 5-year-old girl was referred to us because the cytogenetic blood analysis showed an extra material on the short arms of chromosome 8; Karyotype: 46,XX, add(8)(p22). Proband presents severe mental retardation and dysmorphic features. FISH study shows monosomy 8ptel and trisomy 4ptel inherited from her mother.

To our knowledge there are no other cases described in the literature with the same subtelomeric chromosome rearrangements. We discuss genetic counselling aspects and patients evolution.

P02.181 Terminal osseous dysplasia with pigmentary defects (TODPD): follow-up of the first reported family, characterization of the radiological phenotype, and refinement of the linkage region.

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Terminal osseous dysplasia with pigmentary defects (TODPD) is an X-linked dominant syndrome with distal limb anomalies and pigmentary skin defects. We have previously described this syndrome in several females from a large, four-generation pedigree. The presentation in the affected patients included multiple anomalies, hypertelorism, iris colobomas, punched-out pigmentary abnormalities over the face and scalp, brachydactyly, and digital fibromatoses. The phenotype was highly variable thus suggesting that X-inactivation plays an important role in the expression of the disease. Following our initial description of this condition there have been reports of more cases supporting the initial phenotypic description of this disease. We report the follow-up of this family at about 10 years from the first evaluation. A detailed clinical follow-up and a review of the skeletal surveys suggests that although the most striking features involves the hands and feet, the skeletal involvement is more generalized and affects many other areas.

Our previous linkage analysis has demonstrated mapping to Xq27.3-Xq28. Using a 6,056 SNP array, we have further refined the critical region within the Xq28 region. We have also excluded two candidate genes (*FLNA* and *FAM58A*) mapping in the critical region. The identification of the gene responsible for this rare condition will shed light on the molecular pathways leading to the various congenital anomalies of TODPD and will allow a more accurate genetic counseling to the

affected individuals.

P02.182 Tetraploid/diploid mosaicism - An easily overlooked cause of dysmorphism

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Chromosomal mosaicism not detectable in blood lymphocyte metaphases is an easily overlooked cause of dysmorphism. We present a boy with tetraploid/diploid mosaicism, a type of mosaicism that also remains undetectable upon DNA diagnostic screening on genomic microarrays. Clinical suspicion of the mosaicism is therefore essential.

The boy is mentally retarded (walked at age 2 years, no oral language at age 4,5 years, normal behaviour) with distinctive dysmorphic features: Triangular face, marked hypertelorism, short/downslanting palpebral fissures, posteriorly rotated simple ears with narrow auditory channels, short neck, asymmetric thorax and one café-au-lait spot. He also suffers from early loss of all deciduous incisors due to aggressive periodontitis. Height, weight and head circumference are in the low normal range (2.5-10 centile).

In this case, despite normal blood karyotype 46,XY and normal HR-CGH, a chromosomal mosaicism was suspected because of a „chromosomal appearance“ and slight asymmetry. Fibroblast culture from a skin biopsy revealed non-mosaic tetraploidy (92,XXXX) which was confirmed with interphase FISH. In contrast, FISH investigation of peripheral blood leukocytes and buccal cells did not show signs of tetraploidy. As expected, a haplotype study of DNA from both parents and the boy's fibroblasts indicated that the tetraploidy resulted from mitotic slippage or failed cytokinesis during early embryonic development.

P02.183 The simultaneous presence of α and β thalassaemia alleles: a pitfall for thalassaemia screening

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Haemoglobinopathies are a group of monogenic disorders characterized by reduction of one or more globin chain synthesis or a structurally abnormal globin chain synthesis. Accurate determination of carrier haematological phenotype is quite useful in order to determine the underlying carrier genotype. Although haemoglobin disorders are the best studied (mainly for α - and β -thalassemias), autosomal genetic disorders and haematological phenotypes are not always well correlated with specific gene mutations. The co-existence of different types of mutations in thalassemia genes alter typical haematological patterns and can lead to genotype / prenatal misdiagnosis, especially in cases of 'silent' carriers.

In this poster we present distinct combinations of co-inheritance of alpha, beta and delta thalassemias (alpha and beta, alpha and delta, beta and delta) and how these combinations alter the haematological data. Furthermore we suggest more extended genotype analysis approach especially for couples where prenatal diagnosis is needed. In countries like Greece, where α - and β - thalassemia traits are quite high, additional care must be given in β -thalassemia carriers who could possibly carry a 'well hidden' α -thalassemia severe mutation. Even in cases where amelioration of erythrocyte indices (MCV, MCH) is observed, MCH is not always modified. In couples where one partner seems to be an α -thalassemia carrier and the other a β -thalassemia carrier, in the latter, HbA2 is not practically altered when an α -thalassemia trait co-exists. In such cases, simultaneous analysis of both genes mutations should be carried out in order to achieve safer prenatal diagnosis and more effective/accurate genetic counselling.

P02.184 Townes-Brocks syndrome: report of a first case of Townes-Brocks syndrome with congenital adrenal hyperplasia

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Townes-Brocks syndrome (TBS) characterized by imperforated anus (82%), dysplastic ears (88%) (over folded superior helices and pre-auricular tags) frequently associated with sensorineural and/or conductive hearing impairment (65%), and thumb malformations (89%) (triphalangeal thumbs, duplication of the thumb, preaxial polydactyly and rarely hypoplasia of the thumb).

Renal impairment (27%), including end-stage renal disease (ESRD)

(42%), may occur with or without structural abnormalities (mild mal-rotation, ectopia, horseshoe kidney, renal hypoplasia, polycystic kidneys, vesicouterteral reflux). Congenital heart disease occurs in 25%, genitourinary malformations (36%). Mental retardation occurs in approximately 10% of cases. It is autosomal dominant disease with variability in the severity of expression.

We are reporting a 8 year girl with dysplastic ears, deafness, dysplastic thumbs, small kidneys, history of repaired imperforated anus, and rectovaginal fistula. She is also diagnosed with congenital adrenal hyperplasia with congenital hyperplasia. We believe our patient is the first case of Townes-Brocks syndrome.

P02.185 Imperforate anus, ear and renal anomalies: is the Townes-Brocks syndrome possible?

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Introduction: Townes-Brocks syndrome (TBS) is characterized by the triad of imperforate anus (82%), dysplastic ears (88%) and thumb malformations (89%). Renal impairment (27%) may occur with or without structural abnormalities. Material and method: The authors describe the case of a new-born male referred to our hospital for the proper surgical treatment of a life-threatening anomaly: imperforate anus. Results: The physical revealed right facial nerve paralysis, microtia, and preauricular pit, while the cerebral MRI concluded that the external auditory canal and the middle ear were absent, but with preserved inner ear structures. We also performed several other imaging studies: the abdominal ultrasound suggested crossed fused renal ectopia and the abdominal MRI confirmed these findings; vesico-urethral reflux and renal scarring on the ectopic kidney were found on voiding cystography and scintigraphy scan, respectively; the cardiac ultrasound revealed a ventricular septal defect. Corroborating the obtained data with and the international experience, we included our case in the TBS phenotype. Initially a left colostomy was performed immediately after birth, followed at 8 months of age by a posterior sagittal anorectoplasty (Pena procedure) and the nephrectomy of the ectopic kidney. The latter was performed in an Austrian hospital. The patient had multiple urinary tract infections, which stopped after the second intervention. Conclusions: Genetic testing should be performed (mutation in the SALL1 gene) to avoid confusion due to the absence of thumb malformations.

P02.186 A report of a familial translocation with different clinical manifestaion

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We are reporting a baby boy with abnormal phenotype who is a carrier of an apparently balanced translocation between chromosome 16 and 20. He is the second child of an unrelated couple. The first child shortly after birth was dead due to respiratory infection and being premature. This proband was delivered at 32 weeks of gestation. His birth weight was 1/75 kg. He had low set ears, hydrocephaly, undetected testis, cleft plate, and abnormal hands and feet. He was also dead because of respiratory infection. The karyotype of the baby found to be 46, XY, tr(16;20)(q11.2;p13)mat. Since there are 2 cases of still birth in this family with familial translocation we think it is worth to report that.

Lymphocyte cultures from the patients were set up in RPMI 1640 supplemented with 20% FBS. highresolution chromosome banding was performed in all subject.

P02.187 A case of triploidy (69,XXX) with long survival time

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The liveborn infants with triploidy is a very rare event and death usually occurs within the first hours of life. Triploid cases with a survival of more than two months are infrequent. We report on a liveborn triploid female, born after 36-37 weeks of gestation and who survived 67 days. Chromosomal analysis demonstrated a 69,XXX karyotype with

no evidence of mosaicism. The clinical features are growth retardation, prominent occiput, open anterior and posterior fontanelles, telecanthus, short and asymmetric palpebral fissures, wide nasal bridge, large nose, deep filter, high and narrow palate, microgenia, deformed low set ears, abnormal length all fingers, abnormal dermatoglyphics, long narrow feet, chest deformation, skin grooves in lumbar region, ventricular septum defect and open arterial duct. The placenta was small and noncystic. The proband died of cardiac arrest.

P02.188 Partial trisomy of chromosome 18q21 in a woman led to a full trisomy of chromosome 18 in a fatal embryo

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Introduction: Trisomy18 syndrome, is one of the most common autosomal anomalies. Different candidate critical regions have been proposed for Edwards syndrome extended from proximal 18q12 to distal 18q21. Aim of the study: We report a woman with partial trisomy 18q21 who had 3 early abortions in embryos with full trisomy of #18.

Patients: In March 2008 a couple was transferred to Cytogenetic unit because of a history of 3 early abortions with the embryos having trisomy 18. The husband had a normal phenotype, while the woman 30yr old, had short neck, a finding that was also had her mother who refused a chromosomal analysis.

Methods: Peripheral blood lymphocytes were cultured using standard techniques. Thirty GTG banded metaphases were analyzed (ISCN2005). FISH was done with the 18SE centromeric probe (KREATECH) and LSI MALT1(18q21), which identifies #18 aneuploidy (VYSIS).

Cohybridization was done with Telvysion13q(so)/Telvysion 21q(sg). Two hundred interface nuclei were counted for any single probe in both partners.

RESULTS: The husband had normal results (karyotype/FISH).

The wife had 2 abnormal karyotypic cells and with FISH 10% of her cells were trisomic for 18q21 region.

Conclusion: Pure partial duplication of #18 is a very rare syndrome. In our case it is important that a mother with a partial trisomy of 18q21 critical region aborted 3 embryos with full trisomy #18.

Reports suggest that the critical regions 18q11 and 18q21 are implicated in the etiology of the +18 syndrome and the risk of such interaction errors increase with advance parental age and can happen during the division of reproductive cells in the carrier parent.

P02.189 TSC2-PKD1 gene deletion related to Tuberous Sclerosis and Polycystic Kidney Disease Symptoms

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Tuberous Sclerosis (TS) is an autosomal dominant disorder caused by mutations in TSC1/TSC2 genes. Both are tumour suppressor genes and encode the hamartin (TSC1) and tuberin (TSC2) proteins.

Variable penetrance and a wide clinical spectrum are hallmarks of TS. Genotype-phenotype correlation studies have shown that TSC2 mutations are associated with a more severe phenotype than those found in TSC1. In some cases this affirmation is biased by the presence of alterations that compromise both TSC2 and PKD1. PKD1 is responsible for the Autosomal Dominant Polycystic Kidney Disease (ADPKD) and is located adjacent to TSC2 in tail to tail orientation.

We present here two unrelated patients with no family history of TS and negative for small-mutation screening in TSC1/TSC2. Clinical criteria of patient 1 (3 years) are: Hypomelanotic macules, cardiac rhabdomyoma, renal cysts, cortical tubers and retinal hamartoma. Clinical criteria of patient 2 (22 years) are: Angiofibromas, cardiac rhabdomyoma, renal cysts and cortical tubers. Using MLPA assays, we found a heterozygous deletion encompassing both TSC2 and PKD1 genes in both patients. Patient 1: The deletion encompasses TSC2 E30-E41 and entire PKD1; Patient 2: The deletion encompasses the entire TSC2 and PKD1. Both patients have major criteria of TS and renal cysts which is concordant with TSC2/PKD1 contiguous gene syndrome. We propose that MLPA analysis of the TSC2-PKD1 region should be considered as preliminary screening in patients with PKD and TS criteria.

P02.190 Unusual presentation of tuberous sclerosis complex in neonatal period

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Tuberous sclerosis is severe autosomal dominant hamartosis that affects various organs and systems, predominantly skin and CNS. Most of the symptoms appear later during infancy, such as seizures, skin and bone lesions, as well as mental retardation. Early diagnosis in neonatal period is difficult and can be established if variable dysmorphic features are present.

We report on a male newborn with major facial and body anomalies. It is the third child in a family without the history of chronic diseases. The pregnancy and delivery was normal, with high birthweight. The baby had hemihypertrophy of the whole left side - the body and face, especially the left arm which was immobile. The hands and feet were big, rough with loose redundant skin. The fingers especially on the left hand were large and inflexible. There is a vascular patchy scar-like lesion on the left hemithorax. Ultrasonography of the heart confirmed rhabdomiomas. KTM of the brain was performed at the age of 2.5 months when seizures started, showing existence of tumors inside the third ventricle and one in frontal lobe.

The phenotype of TS in early infancy is variable -from no sign to full blown clinical presentation with hemihypertrophy and skin lesions. The combination of signs of tuberous sclerosis with big rough hands is rather unusual, and can be confused with Proteus syndrome at early age. There are reports of enlargement of a single finger, however this patient have both hands and almost all fingers enlarged. Molecular diagnosis is needed to establish the diagnosis.

P02.191 Germinal Paternal Downstream of *SHOX* Deletion Associated with Post-zygotic Mosaic Maternal X-Chromosome Loss Leading to Nullizygote Langer Mesomelic Dychondro genesis: Implications for Disease Mechanisms and Genetic Counseling

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Mutations or deletions in the *SHOX* gene lead to Langer mesomelic dysplasia (LMD) when present in homozygous form. A new class of deletions was defined recently 30-250 kb downstream of *SHOX* and shown to display enhancer activity to the *SHOX* promoter. In a female with LMD stigmata, monosomy for X-chromosome was estimated at 31% of her lymphocytes. Further molecular studies included the segregation of 17 highly polymorphic microsatellite markers and STRs flanking and including the *SHOX* locus, spanning 328 kb of PAR1 region. A deletion up to 10 kb residing 197 kb downstream of *SHOX* gene was detected, which was germinally transmitted from her clinically unaffected father. This was associated with post-zygotic reduction to nullizygosity of disease-causing region due to mosaic loss of the normal maternal X-chromosome, as shown by fluorescent fragment analysis. Since most cases of LMD with deleted downstream of *SHOX* gene are associated with double heterozygosity for *SHOX* mutation, further studies are required to elucidate the role of the former region in disease etiology. Mutations should be sought in clinically non-affected family related members due to variable expressivity in hemizygous carriers, and cytogenetic evaluation should be considered to detect possible X-chromosome rearrangements underlying the haploinsufficiency for the PAR1, when deletion is detected by molecular analysis. Similarly, when LMD stigmata occur in a Turner's syndrome patient, the possibility of mutations in *SHOX* and the downstream of *SHOX* gene should be explored.

P02.192 Further evidence of genetic homogeneity in Urofacial (Ochoa) syndrome

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The urofacial syndrome (OCHOA; OMIM 236730), is a rare autosomal recessive disease characterized by facial abnormalities, and uropathy. The patients with urofacial syndrome have paradoxical inversion of facial musculature during smile as crying facies and at the same time they present enuresis with neurogenic bladder. This disorder was previously mapped to 10q23-q24 region and no locus heterogeneity has yet been reported. In this study, we report genetic analysis results of a new consanguineous family with six offspring, four of whom were affected. The index case was a 16-year-old girl with an end-stage renal failure for the last three years. She was under continuous ambulatory peritoneal dialysis. In the medical history, there was recurrent urinary infections, incontinence and enuresis. An appearance of crying face during smiling was noticed. Voiding cystourethrogram of the index case showed a trabeculated, diverticulated and low capacity bladder and left high-grade vesicoureteral reflux. In addition, there was bilateral grade 4-5 hydronephrosis in her renal ultrasound. She had five siblings. Three of them had similar medical history and clinical findings. Their mental status and other examination findings were normal. Homozygosity analysis using 250K Affymetrix SNP array (Nspl digestion) revealed a single long homozygote segment of 10Mb in size spanned between 94-104 megabases on chromosome 10q23-q24. The results of this study further support the hypothesis that the same gene on 10q was responsible for all UFS patients from multiple ethnic groups.

P02.193 22q11 deletion screening in patients with palatal abnormalities and suspicion of Velocardiofacial syndrome: preliminary results.

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Velocardiofacial syndrome (VCFS) is the most common syndrome associated with cleft palate and occurs in approximately 1 in 4000 live births. It is caused by a sub-microscopic deletion at 22q11.2, and the main characteristics include congenital cardiac defects, characteristic facial dysmorphic features, hypocalcemia, learning disabilities, developmental delays and palatal abnormalities. The palatal abnormalities occur in approximately 70% of the cases and include cleft palate, submucous cleft palate and velopharyngeal insufficiency. Delayed diagnosis of VCFS is a significant health problem and valid criteria for testing are needed. The aim of this study is to screen 22q11 deletion in patients with palatal abnormalities and clinical suspicion of VCFS. After specific evaluation by a clinical geneticist, 52 patients were selected. We used the SALSA P250 MLPA (Multiplex Ligation-dependent Probe Amplification) kit and followed the manufacturer's instructions. The MLPA results have been confirmed by FISH. We detected 22q11 deletion in 18 patients and a duplication 8p in one patient without 22q11 deletion. Until now, we detected genomic imbalances in 19 of 52 patients (37%). Previous studies that screened 22q11 deletion in patients with palatal abnormalities, without clinical genetics evaluation and specific criteria for patients' selection, found a very low detection rate (0-5%) and concluded that a routine screening for individuals with an isolated palatal anomaly may not be required. Here we demonstrated that the screening for 22q11 deletion in patients with palatal abnormalities, after specific evaluation by a clinical geneticist, could be more feasible, improving the detection rate. **Financial Support:** Fapesp, CNPq, Capes.

P02.194 Locus heterogeneity in hyperkeratosis-deafness syndrome

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Hereditary palmoplantar keratodermas (PPK) are a clinically and genetically heterogeneous group of diseases, which share impaired epidermal differentiation. A particular syndromic form of PPK, PPK with deafness, was first reported in a father and his daughter by Vohwinkel in 1929 (Vohwinkel syndrome). A small number of cases have been described subsequently and most molecularly studied patients were found to carry distinct, heterozygous missense mutation in the GJB2 gene encoding connexin 26. We diagnosed severe PPK associated with congenital deafness in a male and a female patient, who were 4th

degree cousins from one family. GJB2 mutation analysis and haplotype analysis excluded disease-causing mutations in this gene. A SNP array-based genome scan identified a 4-Mb region on 15q26 segregating with the disease under the hypothesis of autosomal recessive inheritance. We are currently recruiting further patients and families with PPK associated with deafness without GJB2 gene mutations in order to identify a gene for this condition using linkage analysis and second generation sequencing of a minimal candidate region.

P02.195 A girl with growth retardation, mental deficiency and unusual face

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We present a 5 year old girl from first uneventful pregnancy and delivery. She was born in 34 gw with birth weight 2000g and birth lenght 40cm. The parents are young, without consanguinity. The child is mentally and physicaly retarded since early infancy. At the age of 5 her lenght is 96 cm (SDS -2.9). The face is dysmorphic- dolichocephaly, short nose, high arched palate, long filtrum, hypertelorism, , short and downslanting palpebral fissures,epicanthus, low placed ears, mild hypertrichosis over the limbs and dorsum. Investigations including GH level, thyroid hormones, EMG, MRI and chromosomal analysis are normal.

Our patient has facial features similar with the children, described by Wiedemann(1989) and Steiner(2000).

P02.196 Thorough evaluation of the cardiovascular system in a large cohort of patients with Williams syndrome

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Williams syndrome (WS) is a complex developmental disorder characterized by congenital cardiovascular disease, dysmorphic facies, mental retardation and infantile hypercalcemia. WS is due to microdeletion of chromosome 7q11.23 involving elastin gene and 25 others. Cardiac involvement with supravalvar aortic stenosis (SVAS) and supravalvar (SVPS) or peripheral pulmonic stenosis (PPS) and even mitral valve prolapse or incompetence (MVP or I) are well known complications that require intervention, often in childhood. However, and even in the absence of these lesions, high blood pressure (HBP) is seen in 50% of patients, and often appears at a young age, and goes often undiagnosed in our experience. The cause of the hypertension is largely unknown, "essential" in most cases, and renal artery stenoses are rare. The central vasculature, including the supraaortic, thoracic and abdominal branches of the aorta have not been extensively explored, and how the progressive thickening of the vessel walls and consequent stenoses of the lumens relate to the development of hypertension is uncertain.

We have evaluated with 24 h ambulatory blood pressure monitoring a cohort of WS patients, and are currently examining the anatomy and function of the heart and vessels with several techniques. The following table illustrates the great frequency of HBP, the variety and consistency of the cardiovascular findings, and the need for monitoring and intervention in these patients.

| | Normal | Abnormal | Major findings | Observations |
|---------------------------------|--------|--|--|---|
| Blood pressure ABPM (41) | 12 | HBP 18 BP>75% 11 | Loss of nocturnal dip 24 Elevated systolic BP 25 | Most undiagnosed |
| Echocardiogram (44) | 13 | 31 | SVAS 19 PPS 6 MVP or I 10 Surgery 4 | Coarctation of the aorta is seen in 4 cases |
| Doppler ultrasound (27) | 13 | 14 | Aortic narrowing, accelerated flows in carotid, renal, abdominal branches | Excellent screening tool |
| Angio CT (15) | 1 | total 14 thoracic 7 abdominal 13 | Midaortic diffuse narrowing Stenoses of the celiac and superior mesenteric No renal stenoses | Defines the extent of the narrowings Diffuse involvement |

P02.197 Phenotypic variability in children with Williams syndrome

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Objective: We present our research regarding the variability of the clinical features of children with Williams syndrome (WS).

Material and methods: 10 patients with clinical suspicion of WS admitted to our Department in the last 3 years were included in this study. All children were evaluated by clinical (dysmorphological, neurological, psychological evaluations) and paraclinical (blood tests, echocardiography, neuroimaging studies) examinations. Cytogenetic studies - karyotype and FISH for 7q11.23 - were performed, confirming the diagnosis.

Results: Our patients (6 boys and 4 girls, age ranging from 6 months to 15 years) showed a clinical phenotype suggestive for WS: distinctive facial dysmorphic features, motor developmental delay with an average walk age of 24 months, failure to thrive, hyperacusis. Nine children had moderate mental retardation, and one child had severe mental retardation associated with congenital cerebral malformation and infantile spasms. Almost all children (8 out of 10) had speech delay, with first spoken words at a mean age of 36 months. Four patients presented cardiovascular problems, and one child had pyloric stenosis. Visuospatial disorientation was present in all tested children. Hypercalcaemia was noted in 4 children, all under the age of 3.

Conclusions: There was a phenotypic variability among our patients, especially regarding walk and speech age. Atypically, the majority of our patients had speech delay. Only 4 children had cardiovascular problems. A particular case was that of the child who associated cerebral malformation and infantile spasms.

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P02.198 High frequency of autistic traits in Williams-Beuren patients.

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Williams-Beuren syndrome (WBS) is characterized by elfin facies, congenital heart disease (supravalvar aortic stenosis), mental retardation and peculiar hyper sociable behavior. It is caused by microdeletion of 1.5 to 1.8Mb in 7q.11.23 region.

We studied 31 WBS patients (20 M and 12 F) and their age ranged from 9 to 26 years (median 14y). The diagnosis of WBS was confirmed by FISH, MLPA or micro satellite markers analysis in all patients.

The objectives were to evaluate cognitive ability, the execution IQ, verbal and total, the frequency of visual-spatial deficits and autistic traits. The tests used were: WISC-III, WAIS-III, Rey Complex Figure and scale of autistic traits (ATA).

All patients had cognitive impairment in all tests, the total IQ ranged from 51 to 86 (median 63): 22 with mild mental retardation, 4 moder-

ate, 4 borderline and 1 on average lower. All patients had marked visual-spatial deficit. The frequency of autistic traits were found in 13/31 patients (41.94%) with predominance in males (10M: 3F). No correlation was found between the size of the deletion and the presence or absence of autism.

Our study reinforces the importance of the systematic assessment of the cognitive function in WBS patients and alert for the presence of a high frequency of autistic traits, opposite of overfriendliness personality typically found in WBS patients. These latter data are preliminary and further studies are necessary to confirm this specific finding in WBS. (FAPESP)

P02.199 Recurrent achalasia in a child with Williams-Beuren syndrome

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Williams syndrome is a multisystem genetic disorder caused by the 1.6Mb hemizygous deletion involving the elastin gene in the region q11.23 of chromosome 7. The phenotype of Williams syndrome is extremely variable but the most common findings include cardiovascular disease, distinctive facies, mental retardation, a specific cognitive profile, endocrine abnormalities, growth retardation and connective tissue abnormalities. Although gastrointestinal difficulties are one of the most constant and prominent finding of the syndrome, including gastro-esophageal reflux (GER), poor suckling, vomiting, constipation, prolonged colic, rectal prolapse, inguinal, umbilical and hiatal hernia, there have been no reports of achalasia in association with Williams syndrome in the literature. We present the case of a boy with Williams syndrome, achalasia and

recurrent postoperative stenosis of the cardia. After Heller myotomy, the boy developed severe restenosis of the cardia with abundant adhesions which repeated after every treatment, five times in periods shorter than one month. Eventually, he developed GER, erosive gastritis and hiatal hernia which led to severe malnutrition and failure to thrive. Although the genetic defect causing Williams syndrome might not be the direct cause of achalasia we suggest that the frequent development of severe restenosis of cardia due to tight adhesions could be the consequence of elastin gene haploinsufficiency and altered structure and function of elastic fibers in esophageal connective tissue. This case highlights the importance of early diagnosis of esophageal motor disorders in childhood which should be included in the differential diagnosis when a child with Williams syndrome presents with dysphagia and/or regurgitation.

P02.200 Molecular analysis of WT1 gene in patients with Frasier and Denys-Drash syndromes in Brazil

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Mutations in WT1 gene have been identified in different syndromes, such as Denys-Drash (DDS) and Frasier (FS). Both DDS and FS are characterized by gonadal and renal abnormalities and predisposition to neoplasia associated with WT1 point mutations. In DDS, mutations act in a dominant negative way and often occur in the zinc finger region abolishing DNA-binding capacity and leading to sex ambiguity, diffuse mesangial sclerosis with chronic renal disease and high incidence of Wilms tumor (WT). The FS presents with dysgenic gonads, male-to-female sex reversal in 46,XY subjects, pubertal delay in both sexes, nephrotic syndrome and focal segmental glomerulosclerosis leading to chronic renal disease and high incidence of gonadoblastoma, but without WT. We report the molecular investigation of WT1 gene in one patient with DDS and two patients with FS. Direct sequencing revealed the most frequent mutation in DDS, the R394W in exon 9. The two FS patients presented with the heterozygous mutations IVS9+4C>T and IVS9+5G>A, respectively. These mutations are known to affect the correct splicing of intron 9, causing different ratios from the normal 2KTS(+):1KTS(-) ratio. Present results are in agreement with those that consider exon 8-9 as a hot spot for mutations in WT1 gene. In addition, the mutation IVS9+5G>A was identified in a female patient diag-

nosed initially as having Nephrotic Syndrome. Frasier Syndrome was considered after finding the mutation and karyotyping as 46,XY. These data highlight the importance of molecular analysis as a complement for clinical diagnosis and genetic counseling in Nephrotic Syndrome associated with sexual disorders.

P02.201 A 130kb deletion in FMR2 may be the cause of developmental delay of one-year-old boy.

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An unstable GCC triplet repeat responsible for folate sensitive fragile site FRAXE in the 5' untranslated region of FMR2 gene is known to cause X-linked mental retardation (XMR). To the best of our knowledge no point mutations or deletions confined to the FMR2 gene has been associated with mental retardation so far as it could be expected in analogy to the FMR1 gene mutations.

Here we report clinical and molecular data of a one-year-old boy with a mild mental retardation and a de novo 130kb deletion of FMR2 gene. Additionally, he presented with minor dysmorphic features and anomalies: up slant palpebral fissures, hypertelorism, epicanthic folds, small nose with a broad prominent base, long philtrum, high palate, crumpled ears helix, short neck, narrow shoulders, and one sided supernumerary nipple. Cytogenetic analysis showed maternally inherited balanced Robertsonian translocation, t(13;14). Methylation studies did not find UPD 14. SNP array (Affymetrix 250K Nspl,) showed a 130 kb deletion which involves exons 2 and 3 of FMR2 gene and spans approximately between base pair X:147454718-147586323 (hg 18). The aberration and the de novo status were confirmed by MLPA analysis. The end of the deletion was predicted to cause a frame shift mutation, resulting in a premature stop-kodon and truncated protein. This suggested a disease-causing nature of the described FMR2 gene deletion. Future molecular screening studies of FMR2 gene on males with XRM may be appropriate and could confirm an alternative mechanism of FMR2 associated mental retardation.

P02.202 Growth deficit and Xq chromosome deletion. Case report

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Aims. To present the diagnosis particularities in a child diagnosed with terminal deletion of Xq chromosome. The patient comes from an endemic area for hypothyroidism. Methods. The authors present a 3 years old girl admitted for further investigations in context of growth impairment. The clinical exam has revealed: impaired nutritional status (weight - 3 SD), short stature (-3 SD) with normal proportioned body, short neck, face dimorphism (elfin face, long philtrum), low inserted ears, legs edema, modified palmar creases, low posterior hairline, hypotonia, mental retardation, hyperactive behavior, motor skills delay and limited vocabulary. The girl was evaluated regarding feet lymphedema and growth impairment etiologies. We have performed laboratory / imagistic investigations and genetic tests. Results. The laboratory investigations have shown normal glucose and lipids metabolism. From endocrinological point of view: normal levels for thyroid and adrenal gland function tests; the growth hormone and insulin-growth factor-1 serum levels were also normal. The buccal smear for Barr bodies test was below normal range. The karyotype investigation has revealed Xq chromosome deletion (Xq26:qter). The parents' karyotypes were normal so we consider „de novo” anomaly for patient. Conclusions. We should consider a karyotype performing for any girl with unexplained short stature correlated with mental disabilities. We should monitor the case regarding the osteoporosis evolution and the possibility of premature ovarian failure onset. It's difficult to appreciate the link between growth failure and mentioned genetic anomaly (SHOX genes responsible for growth retardation were identified on Xp chromosome).

P02.203 ZIC3 mutation analysis in five familial cases of heterotaxy: identification of a new mutation

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ZIC3 gene maps on Xq26 and encodes a zinc finger transcription factor involved in early stages of left-right body axis formation. Mutations in this gene have been already proven to cause X-linked visceral heterotaxy (HTX), namely an abnormal arrangement of thoracic and abdominal organs often associated with complex cardiac malformations. We performed ZIC3 mutation analysis by direct sequencing in five familial cases of HTX. Interestingly, we identified a new mutation (c.1306delC) in exon 1, predicted to result in protein truncation. Specifically, the mutation was found in a male patient presenting with an apparently isolated complex cardiac malformation consisting in right atrial isomerism, single ventricle, pulmonary atresia and bilateral superior vena cava. The sister and the mother of the proband, both experiencing repetitive spontaneous abortions, resulted heterozygous for the mutation. Particularly, the autopsy examination of a 21 weeks male fetus conceived by the sister, revealed a right atrial isomerism, atrio-ventricular canal, double outlet right ventricle, severe hypoplasia and transposition of pulmonary artery, asplenia and dextrogastritis. Our study further confirms the high variability of clinical phenotype in patients with ZIC3 mutations, varying from isolated complex cardiac defects to classic heterotaxy.

J02.1 Prevalence of the isolated hereditary pathology of eyes in Kirov area of the Russian Federation

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According to the world statistics about 5 % of all newborns are born with those or other genetically caused defects from which on hereditary diseases of eyes 30 % are necessary approximately. For the purpose of studying of features of prevalence isolated hereditary ophthalmopathology total inspection of the population of six districts of the Kirov region (Nemsky, Sunsky, Uninsky, Shabalinsky, Bogorodsky, Svezhinsky). Size of the investigated population was 54607 persons. The ethnic structure of examined sample on 95 % was presented by Russian. 94 patients with the isolated hereditary pathology of eyes were totally revealed. Research was lead according to elaborated in laboratory of genetic epidemiology of Research Centre for Medical Genetics of the Russian Academy of Medical Science the report, allowing to reveal and clinically to diagnose all spectrum hereditary ophthalmopathologies. Prevalence of the isolated hereditary pathology of eyes (autosomal dominant, autosomal recessive and X-links) in the surveyed districts of the Kirov region has made 1:581 the person (from 1:966 the person in Uninsky district to 1:273 the person in Svezhinsky district). At inspection it has been diagnosed 26 diseases, including a isolated microphthalmia with coloboma, the ocular coloboma, a corneal endothelial dystrophy, a microspherophakia, a congenital cataract, the bilateral optic nerve hypoplasia, a retinitis pigmentosa, Best vitelliform macular dystrophy, Wagner vitreoretinal degeneration, the choroideremia, a hereditary primary glaucoma. The etiology (DNA-analysis) and clinical polymorphism separate forms of a hereditary pathology of eyes were studied.

P03 Cytogenetics

P03.001 10q24 duplication: 10 new cases identified on array-CGH allow prenatal diagnosis and broadening the clinical spectrum

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Ectrodactyly or split hand-split foot malformation (SHFM) is a rare condition that occurs in 1 in 8500-25000 newborns and accounts for around 15 % of all limb reduction defects. SHFM is clinically heterogeneous and can be either isolated, associated with other malformations or part of syndromic entities. This condition is usually inherited

in an autosomal dominant manner and several loci have been identified. Among them, SHFM3 has been located on 10q24 and the naturally occurring *Dactylaplasia* mouse is the animal model for SHFM3 in humans. Recently, 0.5 Mb tandem genomic duplications at chromosome 10q24 involving at least the *DACTYLIN* gene have been found in SHFM3 patients. No point mutations in any of the genes residing within the duplicated region have been reported so far, and it is still not clear how this rearrangement leads to the SHFM3 phenotype. Indeed, complex alterations of gene regulation mechanisms that would impair limb morphogenesis are likely.

We report on one case of split hand-split foot malformation identified during pregnancy, 5 cases of typical SHFM, 3 monodactylies and 1 femoral duplication for which array-CGH was performed on a research project basis on limb malformations. 10q24.31q24.32 duplication (321-637 kb) was identified in all cases, comprising at least the *DACTYLIN* gene. All cases were isolated and non-syndromic.

To our knowledge, this is the first prenatal report of 10q24 duplication in SHFM, as well as the first cases of monodactylies or femoral duplication associated with this cytogenetic anomaly.

P03.002 First description of a patient with a 15q13.3 homozygous microdeletion and epileptic encephalopathy, retinopathy and choreoathetosis

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The increasing use of array-CGH allowed identification of novel microdeletional syndromes. Patients with 15q13.3 microdeletions present with relatively consistent breakpoints at BP4 and BP5, including the *CHRNA7* gene. The phenotypic spectrum is large, ranging from mental retardation with dysmorphic features, epilepsy, neuropsychiatric disturbances, to absence of anomalies. We describe the first case of homozygous 15q13.3 microdeletion in a 6-year-old boy. Both parents carried heterozygous microdeletion and have mild mental retardation. Severe hypotonia were evidenced since birth and abnormal eye movements at 3 months of life led to the diagnosis of rod-cone dystrophy. Pharmacoresistant epilepsy appeared at 3 years of age. At 6 years of age, he had mild dysmorphic features with normal OFC, poor head control, blindness, partial deafness, choreoathetosis responsive to Xenazine®, absent language and atonic seizures. Cerebral MRI only showed an arachnoid cyst. Metabolic screening and nerve conduction studies were normal. Although electroencephalography was not in favour of ceroid lipofuscinosis, a skin biopsy was performed and showed curvilinear-like cell inclusion bodies. Molecular and enzymatic assays ruled out CLN I and II. The 15q13.3 homozygous microdeletion was confirmed by quantitative PCR. The association of convulsive encephalopathy, retinopathy and choreoathetosis is unusual and suggests array-CGH analysis in such cases. The severity of the reported clinical features argues in favour of the pathogenicity of the heterozygous microdeletion.

P03.003 Further delineation of the 17p13.3 microdeletion distal to PAFAH1B1: 4 additional patients

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Background: The Miller Dieker syndrome (MDS, MIM 247200) is characterized by lissencephaly, mental retardation and facial dysmorphisms. The phenotype is attributed to haploinsufficiency of two genes present in 17p13.3 region: PAFAH1B1 and YWHAE. Isolated PAFAH1B1 defect

causes lissencephaly. Small 17p13.3 deletions distal to *PAFAH1B1* but including *YWHAE* result in facial dysmorphisms, growth retardation, cognitive impairment, and variable structural abnormalities of the brain without lissencephaly.

Objective: We describe clinical, neuroradiological and molecular data on four patients with a 17p13.3 deletion distal to *PAFAH1B1* involving *YWHAE*.

Results: All patients presented with mild or moderate developmental disorder and pre and/or post-natal growth retardation. Patients A and C had macrocephaly and leucoencephalopathy with Chiari type 1 malformation for patient A and with paraventricular cysts for patient C. Patient B had patent ductus arteriosus and pulmonary arterial hypertension. Patient C had unilateral club foot. Patient D had enlarged Virchow Robin spaces, microcornea and chorioretinal and lens coloboma. Array-CGH revealed *de novo* terminal 17p13.3 deletions for patient A and B, and showed interstitial 17p13.3 deletions of 1.4 Mb for patient C and of 0.5 Mb for patient D.

Conclusion: Our patients confirm that 17p deletion distal to *PAFAH1B1* have a distinctive phenotype: mild mental retardation, moderate to severe growth retardation, white matter anomalies and developmental defects including Chiari type 1 malformation and coloboma. Our patients contribute to the delineation and clinical characterization of 17p13.3 deletion distal to *PAFAH1B1* and highlight the role of the region containing *YWHAE* in brain and eye development and in somatic growth.

P03.004 Genotype-phenotype delineation of terminal deletions 18q demonstrating high phenotypic variability.

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Constitutive heterozygous 18q deletion is one of the most common segmental aneuploidies compatible with life and usually leads to variable phenotypes. We reevaluated ten patients with 18q deletion and clinical features of different degrees of severity, using molecular cytogenetic techniques in order to better define the genotype-phenotype correlations. FISH with bacterial artificial chromosomes (BACs) revealed three different breakpoints: at 18q21.31 (3 cases), 18q21.33 (5 cases) and 18q22.2 (2 cases), showing genomic losses of 11.3, 17.6 and 21.7 Mb, respectively. In one of the patients, the location of the breakpoint was refined by array-CGH, showing absence of associated genomic abnormalities. Genotype-phenotype correlation demonstrated high phenotypic variability and no evidence that the severity of the phenotype was associated only with the size of the deletion. Genomic analysis showed that the deleted region of 21.7 Mb encompasses several diploid and non-diploid genes that may be associated with different degrees of clinical manifestations. However, the role that each of these genes plays in producing the specific phenotype features is still unknown. Molecular identification of these deleted regions and candidate genes may contribute to establish a predictive phenotype map, as well as a better understanding of the genomic complexity of 18q deletions. (Financial support: FAPESP, Brazil).

P03.005 *De novo* deletion of 1q24.3-q31.2 in a patient with severe growth retardation

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Interstitial 1q deletions have been classified into three groups: proximal deletion (1q21-22q25), intermediate (1q24-25q32), and distal (1q42-43qter). To our knowledge, only seven deletions have been characterized by molecular methods. We report a patient with 1q24.3-q31.2 deletion, which was thoroughly analyzed by high density SNP array as well as junctional cloning. The proband is a 3 1/3 year-old boy. He was born at 35 weeks of gestation by cesarean section due

to fetal distress between nonconsanguineous 39-year-old mother and 37-year-old father. Intrauterine growth retardation was suspected at 27 weeks of gestation. Birth length was 36 cm (-3.7SD), weight 1324 g (-3.8SD), and head circumference (OFC) 29 cm (-1.5SD). At age of 3 1/3 years, his length is 65 cm (-8.5 SD), weight 5890 g (-4.5 SD), and OFC 41.6 cm (-5.5 SD), indicating obvious pre- and postnatal growth retardation. Multiple anomalies were recognized. Serum TSH, fT3, and fT4 were normal. IGF-1 and IGFBP-3 were low and GH stimulation test by clonidine and L-DOPA indicated GH deficiency. Serum antithrombin III (AT III) was also at a low level. GTG-binding chromosome analysis of the patient's blood lymphocytes demonstrated 46,XY, del(1)(q23q25), t(4;11)(q31.3;q21). Parental karyotype was all normal. Affymetrix GeneChip Human SNP array 6.0 (Affymetrix, Santa Clara, CA) clearly demonstrated 1q24.3-q31.2 deletion. Deletion junction was determined at nucleotide level. The translocation, t(4;11)(q31.3;q21) was also carefully analyzed. Genotype-phenotype correlations will be discussed.

P03.006 A new case of 21q interstitial deletion detected by array CGH.

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We describe a 9-year-old boy with proportionate short stature, microcephaly and profound developmental delay. Since 1 year of age he presented with seizures and hearing loss. He has peculiar morphologic facial features: long palpebral fissures, prominent and convex nasal bridge with underdeveloped alae nasi; short philtrum with thin upper lip and long ears. He has intermittent exotropia, no speech and frequent falls during walking.

We performed array CGH (Comparative Genomic Hybridization) which showed an interstitial deletion of chromosome 21q, encompassing bands 21q22.13 and 21q22.2.

This deletion was confirmed by FISH analysis.

The deletion extent is about 5 Mb, and in this region several genes are harboured.

The role of the potential genes in relation to the patient's phenotype is discussed.

P03.007 Variable phenotype in familiar unbalanced subtelomeric translocation 46,XX.mlpa 17psubtel (P070)x3, 22qsubtel (P070)x1

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Background. Screening for rearrangements of the subtelomeric region remains to be a valuable tool for research in idiopathic mental retardation. About 50% of unbalanced translocations are parentally inherited. Some subtelomeric abnormalities are not believed to be associated with a discernible phenotype, including dup(17p) and del(21q). We report on a familiar double imbalance of these particular subtelomeric regions.

Case report. B.M. is the child of a couple with the history of special educational needs in the mother and her younger sister, and dyslexia in the child's father. Her neonatal period was uneventful. Later on motor and particularly speech development were markedly delayed. At 30 months the girl showed normal seize, brachycephaly with normal OFC and midface dysmorphism with square nasal tip. She was able to speak single words. Neuroimaging was normal.

Lab investigation. The patient and her parents underwent standard karyotyping and subtelomeric screening using MLPA and FISH. An unbalanced subtelomeric rearrangement involving 17p and 21q was found in the patient and her mother.

Discussion. Identification of subtelomeric imbalances is an important contribution in diagnosing patients with mental retardation and thereby enabling proper genetic counselling. In unbalanced translocations, the derivative chromosome most often shows both a duplication and a deletion. While several conditions eg monosomy 1p36 lead to a straightforward clinical diagnosis other chromosomal imbalances remain are found in which causality of the aneusomy remains questionable. Isolated subtelomeric 21q deletion was reported only exceptionally, but subtelomeric 17p duplication never before. Investigation of other family members might certify the genotype-phenotype association.

P03.008 Tetralogy of Fallot due to 22q11.2 deletion not including *TBX1***

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Conotruncal heart defects in 22q11.2 deletion syndrome are thought to be due to *TBX1* haploinsufficiency.

We present a patient with tetralogy of Fallot (TOF), bilateral embryo-toxon posterior and learning problems, but no other features of 22q11.2 deletion syndrome. MLPA analysis showed a small 22q11.2 distal deletion between Mb position 19.266-20.130, including the *SNAP29*, *CRKL*, and *LZTR1* genes, but not *TBX1*. Haploinsufficiency of *Crkl* in mice can cause defects of the cardiac outflow tract, whereas compound heterozygosity for null mutations of the *Crkl* and *Tbx1* genes results in a much greater frequency of cardiovascular defects than that generated by heterozygosity of *Crkl* or *Tbx1* alone¹.

This suggests that conotruncal heart defects in 22q11.2 deletion syndrome might be due to epistatic effects of genes in the proximal deletion region (*TBX1*) with genes in the distal deletion region (*CRKL*?). Furthermore, intragenic mutations in *CRKL* might cause conotruncal heart defects.

¹ D. Guris et al., Dev. Cell. 10:81-92, 2006

P03.009 A unique case of three presumably unrelated chromosome abnormalities (47,XXY, 1p36 deletion and mosaic tetrasomy 12p) in a child with severe malformations showing necessity of several molecular cytogenetic techniques per diagnosis

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Multiple constitutional chromosome abnormalities are extremely rare in newborns and generally manifest either as co-occurrence of gonosomal and autosomal aneuploidy or aneuploidy and a structural rearrangement. Here, we report on a unique case of a child with severe malformations with three presumably unrelated chromosomal imbalances. After standard karyotyping by GTG banding, non-mosaic 47,XXY karyotype (Klinefelter syndrome) was established. Since phenotypic manifestations were far from those of Klinefelter syndrome, we performed high-resolution comparative genomic hybridization (CGH). Metaphase CGH has identified a microdeletion of 1p36.22p36.23 and confirmed the presence of additional chromosome X. The confirmation of 1p36 microdeletion was further made by 1Mb-array-CGH. In addition to deletion spanning 1p36.22p36.23 region and additional chromosome X, array CGH detected partial aneuploidy involving the whole short arm of chromosome 12 in 24% of cells. After FISH with DNA probes for chromosome 12, we were able to demonstrate that it was a mosaic tetrasomy 12p present in 12% of cells. The boy presented with increased height (a feature of Klinefelter syndrome), severe mental retardation, developmental delay and hypotonia as well as dysmorphic features characteristic for both 1p36 deletion syndrome and Pallister-Killian syndrome (mosaic isochromosome 12p). Looking through the available literature, we have not found cases with up to three simultaneous and unrelated constitutional chromosome imbalances. It is noteworthy that each technique applied revealed each "new" abnormality. Therefore, to provide correct diagnosis, one molecular cytogenetic method is not enough, even though some of them (i.e. array CGH) provide for high-resolution genome screen.

P03.010 49,XXXXY and abnormalities in brain white matter

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Introduction: 49,XXXXY is a rare sex chromosomal aneuploidy with a distinct phenotype and mental retardation. We present a patient with this karyotype and abnormalities in brain white matter.

Clinical case: Male, born at term to a 38-year-old gravida 4, healthy and nonconsanguineous mother. No family history. Normal pregnancy and delivery. Apgar 8/9. Birth weight 3750 g (90th centile), length 51 cm (75th-90th centile), head circumference 34.5 cm (75th centile). Physical examination at birth: hypertelorism, flat nasal bridge, upslanting palpebral fissures, low-set ears with prominent auricles, cleft palate, small penis and testicles inside hypoplastic scrotum. Other findings: patent ductus arteriosus and atrial septal defect, hypotonia, lax joints, pes cavus. Outcome: developmental delay specially speech, febrile seizures, asthma, pneumonia, shy and friendly personality, microcephaly (head circumference <3rd centile at 6 1/2-year-old), no short stature (length at 90th-97th centile at 6 1/2-year-old), weight at 50th centile. Karyotype: 49,XXXXY. Brain magnetic resonance imaging at 2 1/2-year-old: multiple extensive bilateral foci of hyperintensity signal in the periventricular and subcortical white matter, mainly in the left parietal and frontal lobes (T2 and Flair), enlargement of the ventricular system, asymmetrical brain volume loss.

Discussion: We think that our patient confirms data of the few cases reported in the literature with brain magnetic resonance imaging, and we must consider changes in cerebral white matter a specific finding in 49,XXXXY patients.

P03.011 7q36.1 monosomy as a result of the intrachromosomal rearrangement

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The patient is a 1,3-year-old boy with developmental delay and distinctive facial features. He is the only child of healthy non-consanguineous parents, born at term by normal delivery after an uneventful pregnancy at term by normal delivery with a 2400g birth weight and length of 45cm. The child suffered from intrauterine infection, poor feeding, allergy, dermatosis and constipation. Clinical examination revealed microcephaly, flat occiput, right eye diminishing and ptosis, strabismus, hypospady, hollow breast, dental (incisor) anomaly, muscular hypotonia, intestinal motility disorders and possible cardiac defect (suspected due to muffled heart sounds and systolic murmur). The patient showed profound psychomotor developmental delay with IQ 47.

Standard karyotype of the proband revealed a de novo intrachromosomal insertion in chromosome 7. Peripheral blood DNA from the proband and both parents were analysed using the Affymetrix Genome-Wide SNP Array 6.0 platform. Copy Number and Loss of Heterozygosity data were generated using the Affymetrix Genotyping Consol 4.0 software and showed a 9,8Mb deletion in 7q36 (chr7:149026058-158812470) in the proband's genome. This deleted region includes a number of genes (e.g. Sonic Hedgehog (SHH), KCNH2 and NOS3 genes) which can be involved in causing the patient's phenotype. Particularly, minimal manifestation of the SHH gene are microcephaly, ptosis, sacral agenesis, tethered cord. Our patient shares some of these characteristics. The SNP microarray shows chromosomal imbalances at a high resolution; this makes it possible to pinpoint the genes affected by the rearrangement and thereby link these genes to the clinical manifestations in the patient.

P03.012 Association of genetic variants of ABCA1 gene with CIII and total cholesterol level in Tehranians with or without Combined HDL/LDL-Cholesterol Phenotype

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Introduction: Studies showed that plasma LDL- and HDL-cholesterol taken jointly provide a suitable phenotype because they comprise a complex trait, reliably measurable, and of considerable epidemiological and clinical interest. In the present study, we investigated the effects of the ABCA1 polymorphism on lipid related variables in Iranian individuals with or without Combined HDL/LDL-Cholesterol Phenotype.

Method: Independent samples of normolipemic subjects from Tehran lipid and glucose study populations (78 male and 121 female) were selected for a case-control-study (high LDL/low HDL versus low LDL/high HDL) with Apo B genotypes as independent factors. Total cholesterol,

triglyceride, HDL-C, LDL-C, Apo AI, Apo B, Apo AIV, Apo CIII and CRP concentration were measured. A segment of the mentioned gene with PCR was amplified and the polymorphism with RFLP (XagI) revealed. Result: In two groups of Combined HDL/LDL-Cholesterol Phenotype the presence of R phenotype increase the Apo CIII concentration (R= 159 ± 73 mg/ml vs. K = 123 ± 44 mg/ml, P<0.042) and CRP concentration (R: 707 (1.2, 2.2) vs. K: 1070(1.9, 2.1), P<0.048) in low HDL-C and high LDL-C group. Also the R phenotype increase total cholesterol concentration (R: 174 ± 37 mg/ml vs. K: 156 ± 26 mg/ml P<0.042) in high HDL-C and low LDL-C group. Conclusion: Two groups of combined HDL/LDL-cholesterol phenotype can be a good predictive for analysis the interaction between the AB-CA1 polymorphism with lipid and related variables such as apoCIII, CRP and cholesterol level in normolipemic subjects.

P03.013 Validation analysis of a 60K customized high resolution CGH-array reveal new genomic imbalances associated with well-defined disorders

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Background: Submicroscopic genomic imbalances underlie well-defined microdeletion and microduplication syndromes and contributes to general developmental disorders. Array Comparative Genomic Hybridization (a-CGH) is a powerful molecular cytogenetic tool to detect genomic imbalances and study disease mechanism and pathogenesis. We report the design and validation of a focused high-resolution oligonucleotide-array CGH assay for clinical laboratory diagnosis of genomic rearrangements, referred to as KaryoArray™.

Methods: We selected 60-mer oligonucleotide features from Agilent's eArray_v4.0 probe library in a custom high resolution format of 8x60K. The average density of pathogenic regions is 7 Kb. KaryoArray™ covers more than 350 clinically relevant regions.

Results: To evaluate the array, we blindly tested 102 samples. Genomic imbalances had previously been detected in 52 of the 102 samples by karyotyping, FISH or MLPA. The remaining 50 samples were sex-matched samples from healthy individuals as negative controls for validation. Focused array CGH detected all known abnormal regions in 52 validation samples with more than 99% concordance and yielding most precise breakpoint boundaries. Interestingly, Karyoarray™ was able to detect new rearrangements in 5/52 cases (9.62%) associated with known genetic disorders.

Discussion: This focused aCGH assay is a flexible, and robust method to diagnose genetic disorders associated with genomic imbalances. Karyoarray™ offers advantages over currently available platforms in the clinical diagnostic laboratory, overcoming many of the limitations of conventional gold standard routine techniques for the analysis of genomic rearrangements. In addition, it was able to identify cryptic submicroscopic imbalances not previously detected with the standard routine techniques in 9.62% of the patients.

P03.014 Detection of pathogenic copy number variation in an adult population with intellectual disability and psychiatric disorders.

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Adults with unexplained intellectual disability (ID) have not been systematically addressed for genetic study. We report our experience testing 94 adult patients affected by mild intellectual disability associated with psychiatric disorders and minor dysmorphic features, recruited as outpatients from Psychiatric services and referred to a clinical geneticist for their evaluation. Patients had never undergone previous genetic evaluation. Genetic analysis included karyotype (800 G-bands), specific molecular analysis to confirm the suspicion of a syndrome, and subtelomeric MLPA (P036 and P070 kits from MRC Holland) and high-resolution aCGH (Agilent 400K).

A genetic imbalance was detected in 25 cases (27%). A chromosome abnormality was observed in 6 cases (two deletions, two balanced reciprocal translocation and two derivatives). Specific analysis re-

vealed five cases of fragile-X syndrome and eight cases with a microdeletion syndrome. Subtelomeric MLPA detected one case with del(10q)+dup(15q) and one case with del(21q)mat. aCGH helped further identifying pathogenic genomic imbalances in three cases (dup(Xp), dup(15q) and del(15q)). Additionally, aCGH identify cryptic rearrangements in two cases with a chromosome abnormality: a dup(Yq) in a male with severe ID and an X chromosome complex rearrangement combined to other abnormalities in a women with moderate DI. Among these patients we found impulse control disorder and schizophrenia as the relevant psychiatric illness. No malformations and medical problems but craniofacial dysmorphism were diagnosed. Our preliminary results show a very high prevalence of genomic imbalances amongst adults with intellectual disability, psychiatric problems and dysmorphia.

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P03.015 Increased aneuploidy and abortive DNA replication associate with neurodegeneration in Alzheimer's disease

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It has been repeatedly debated whether abortive DNA replication leading to gross genomic imbalances (i.e. aneuploidy or tetraploidy) is associated abnormal aging in Alzheimer's disease (AD). This suggests that a relationship, if exists, between genetic or genomic instability, abnormal aging and neuronal death in the AD brain requires additional investigations. Recently, chromosome missegregation producing mosaic aneuploidy of whole chromosomes 21 has been discovered to play a role in the AD brain pathology. To get further insights into this phenomena in the AD brain, we have analyzed aneuploidy and abortive DNA replication in neuronal cells of postmortem AD brain samples using molecular neurocytogenetic approaches (interphase MFISH, quantitative FISH, interphase MCB FISH, and FISH-based DNA replication assays). Aneuploidy was observed in 2-15% of neural cells and replicating DNA signals (singlet/duplet) were present in 0.4-3.7% of neural cells of hippocampus and prefrontal cortex of the AD brain. The frequency of abnormal neural cells (aneuploid and abnormally replicated) affecting different chromosomes was higher in the hippocampus and prefrontal cortex (brain areas which are affected by the neurodegeneration) as to the cerebellum (brain area which is less affected by the neurodegeneration). According to our data, genetically unstable and aneuploid neurons may demonstrate abortive cell cycle re-entry and DNA replication stress the diseased brain. These data indicate that somatic genome instability may be considered as the main genetic mechanism of neuronal death and contributes to the pathogenesis of AD. Supported by Philip Morris USA, Inc.

P03.016 A paternity testing showing two related females with XY at the amelogenin locus

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Amelogenin genes called AMEX and AMEY are located in chromosomes X (Xp22.1-Xp22.3) and Y (Yp11.2) respectively. They show a big homologous regions allowing simultaneous amplification with a same couple of primers. However, they have some differences in size and sequence permitting their use as a sex-typing marker, especially with paternity tests to define female and male samples.

We identified during a routine parentage testing two females; mother and daughter carrying the male and female variants of the amelogenin gene. This is in favor of mendelian inheritance of Y chromosome in the context of female persons. Haplotype analysis of X chromosome showed the presence of two alleles in each locus. The mechanism of this outcome stills unclear. One possible explication could be a gene conversion event occurred in the male germ line from whom the mother is descended. It may be also possible that an unbalanced recombination event has occurred. So that, the obtained proband's X carries both X and Y chromosome copy of the amelogenin locus. More molecular and cytogenetics investigations should clarify this situation.

P03.017 Universal reference samples for diagnostic copy number variation analysis

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Genomic microarrays are widely used in clinical laboratories to identify copy number variants (CNVs) throughout the human genome. CNVs are identified by comparing hybridization signals; sample over reference. The results are therefore affected by the choice and quality of the reference material (RM) used. As yet, no approved CNV RM is available for clinical use. Furthermore, there is a need for RMs in which the CNV profile has been established by several methods, to assist in the evaluation of existing CNV technologies and the development of new ones. We surveyed clinical array CGH labs and found that they use RMs which do not meet the criteria required for clinical diagnostics. We investigated the suitability of existing FDA-approved genomic DNA RMs for pharmacogenetic tests, supplied by ParagonDx, for this purpose. Three male and three female DNAs, plus pools of male and female DNAs were analysed using four microarray platforms (Affymetrix, Agilent, Perkin Elmer and Roche Nimblegen). The performance of these samples was compared to pooled DNAs available from Promega, commonly used in aCGH. The use of ParagonDx DNAs as RMs yielded improved results compared to the Promega DNAs, based on a range of quality measures, for example Nexus QC (measure of noise); ParagonDx DNAs had an average QC of 0.022 vs. Promega 0.057. We conclude that the widespread use of ParagonDx DNAs may improve the consistency of results in clinical applications of aCGH testing. Furthermore this reference set provides well-characterised samples for the evaluation of new CNV detection platforms and technologies.

P03.018 New tools in the cost-efficient detection of pathogenic chromosomal aberrations in patients with MR/MCA by array-CGH.

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Array-based comparative genomic hybridization has become an indispensable tool in the hunt for submicroscopic chromosomal aberrations in patients with MCA/MR. The interpretation of array results is complicated by the discovery of neutral copy number variants (CNVs), that constitute some 5 % of the genome. Thus, there is a need for procedures that (cost-) efficiently distinguish pathogenic from neutral CNVs. An important step in this process is distinguishing common (inherited) CNVs, which are more likely to be neutral, from *de novo* CNVs, which are more likely to be pathogenic. We have implemented an oligo-array platform (Agilent, Santa Clara, USA) for the screening of patients with MR/MCA. For an efficient interpretation of the data, we initially performed trio analyses in which the parents of the patient are hybridized with opposite dyes on one array. To facilitate the individual analysis of each parent, separate 2logR files for the parents are subsequently generated using a home made software package that creates export files that can be uploaded into several commercial software platforms. This approach constitutes a fast way to determine the *de novo* nature of the aberrations that are seen in the patient. An adaptation of this software has also enabled us to analyse six patients on one 4-plex 180K array slide, making our diagnostic tests even more cost-efficient. A database of local CNVs, created with the data of 800 healthy parents, has proven to be of high value in the analysis of patients for whom parents were not available.

P03.019 Array-CGH characterization of three patients with deletion 22q13

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Subtelomeric chromosomal rearrangements are believed to be a common cause of mental retardation. Their prevalence is about 5-7%. Because of their small size (under 5Mb) these subtelomeric aberrations are undetectable by conventional G-banding method. New molecular technologies such as MLPA, FISH and array-CGH have been adapted for subtelomeric or genomic screening in patients with idiopathic men-

tal retardation. In surveys of subtelomeric screening, deletion of 22q13 is the second most common subtelomeric deletion, after deletion 1p36. The prevalence of 22q13 deletion hasn't still determined.

The 22q13 Phelan-McDermid deletion syndrome is characterized by mild-to-moderate range of mental retardation, global developmental delay, absent or severely delayed speech, decreased perception of pain and autistic-like affect. Approximately 75% of deletions are simple (terminal or interstitial), about 25% are complex (as a result an unbalanced translocation).

We describe 3 patients including into subtelomeric screening using MLPA technique. In all cases the subtelomeric screening detected the deletion 22q13. In patients 1 and 2, both with normal karyotype, simple terminal deletion was found at 22q13. In patient 3 with no cytogenetic finding, deletion 22q13 and additional duplication of terminal part of 17p were revealed. The size of every pathogenic microdeletion was determined by means of a high-resolution oligonucleotide-based array-CGH. Our study has not shown significant relationship between the clinical features and the deletion size.

This study was supported by VZ MSM0021622415.

P03.020 Array-CGH characterization of a constitutional complex rearrangement with two 4q and one 1q interstitial deletions in a child with multiples congenital anomalies.

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Constitutional complex rearrangements (CCRs) are considered to be very rare, but the use of molecular techniques like array-CGH is disclosing an increasing amount of CCRs, because they allow a more accurate characterization of the chromosomal rearrangements.

We report the molecular characterization of a female newborn with intrauterine growth retardation and multiple congenital anomalies, including unusual craniofacial features (wide anterior fontanelle 6x3 cm., bitemporal narrowing, hypertelorism with narrow palpebral fissures, dysplastic ears, cleft lip and palate, and micrognathia), congenital heart disease (VSD and ASD), laryngeal stenosis, and a sacral S2 hemivertebra. She developed respiratory distress that progressively deteriorated over the first few days of life, eventually leading to her demise on day three. The post-mortem examination revealed agenesis of the gall bladder in addition to the previous findings.

Pre- and postnatal karyotype were both normal. A standard 44K oligo array-CGH (Agilent Technologies) showed three deletions:

- 1- a 1q23.1q23.3 4Mb deletion
- 2- a 4q21.22q21.3 3.2Mb deletion
- 3- a 4q22.3q23 5.8Mb deletion

Both deletions on chromosome 4 were separated by a 9.4Mb normal region.

Parents' karyotypes and arrays-CGH were strictly normal without any evidence of the previously mentioned findings in their baby.

The molecular mechanisms predisposing to genomic instability and leading to CCRs remain unknown. These mechanisms could involve multiple double strand breaks (DSB) followed by non-homologous end joining (NHEJ). Detailed studies at the molecular level of these CCRs will contribute to the identification of structural DNA elements which might predispose to such complex rearrangements.

P03.021 A duplication of the chromosomal region 7p14.1 involving the GLI3 gene is associated with mental retardation

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We report a 60 years old male affected by severe mental retardation who is carrier of a 1 Mb duplication within the region 7p14.1 (from 41.246.668 to 42.408.368) detected through array-CGH (Human Genome CGH Microarray Kit 4 x 44K, Agilent). The duplicated region involves the genes INHBA and GLI3. As far as we know, duplications of GLI3 have never been previously reported. The haploinsufficiency of GLI3 is related to well known conditions, like Grieg cephalopolysyndactyly syndrome, Pallister-Hall syndrome and Acrocallosal syndrome, and this clearly suggest the gene is sensitive to dosage. It would be

therefore reasonable to speculate that duplications as well as deletions of this gene can be involved in human disease.

The patient never developed speech and always had a significant gait impairment. No malformation, sensory impairment, seizure or medical problem have been reported. He developed Myeloid Chronic Leukemia 2 years ago.

He has a positive family history for mental retardation. One of his first cousins is affected by mild mental retardation: interestingly he carries a 14 Mb duplication of the region 2p16.2p22.1 (from 40.885.368 to 54.807.891). The parents of the patients are dead, the siblings have normal chromosomes and the FISH analysis didn't show any subtle imbalance within those regions.

P03.022 The role of array-CGH in syndromic microdeletion identification: case report of a 22q11.2 deletion

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Herein, we present the case of a 16 years old girl with dysmorphic features and severe mental retardation referred for cytogenetic investigation as a consequence of difficulties in establishing a diagnosis.

The patient was the first child of healthy, nonconsanguineous parents, born after an uneventful pregnancy - though with polyhydramnios-, by normal delivery, with a small for date birth weight (2000 g), nourishing difficulties in the first year of life, failure to thrive, and retarded neuropsychomotor development. The clinical evaluation showed: short stature, dysmorphic features (small malformed ears, microphthalmia, prominent nasal bridge, prominent mandible, microstomia, thick lips, long fingers, bilateral camptodactilia of the 5th finger, bilateral covering of the 2nd toe by the 3rd toe), dorsal kyphosis, severe mental retardation, speech delay, hyperkinesia. The cerebral CT scan was normal.

Classical cytogenetic and molecular investigations were carried out using peripheral blood lymphocytes. The GTG-banded karyotype was normal, unevocative of any genetic syndrome. However, array-CGH (44K, Agilent) indicated a 2.3 Mb deletion on 22q11.21, subsequently confirmed by FISH with T-Box1 probe (Kreatech).

The above described region is characteristically deleted in the velocardiofacial syndrome (VCFS) that covers a wide spectrum of more than 200 physical manifestations, including palate and cardiac anomalies. Our patient's phenotype, with prominent nasal bridge, prominent mandible, camptodactilia, dorsal kyphosis, severe mental retardation was not particularly suggestive for VCFS, therefore no specific investigations were performed at diagnosis. Ultimately, the array-CGH was decisive in unravelling the underlying genetic abnormalities.

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P03.023 Cytogenetic abnormalities found among the autism patients in south India

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Autism is a neurodevelopmental disorder characterized by clinical, etiologic and genetic heterogeneity. It is often associated with other conditions, such as disorders of the CNS (tuberous sclerosis), developmental delay, attention deficit, epilepsy, and anxiety and mood disorders. The prime aim of the present study was to identify the chromosomal alterations found among autism patients in Coimbatore region. In order to investigate the possible cytogenetic damage to the autism patients, a G-banding method was carried out on the lymphocytes of 26 experimental samples and equal number of controls. In the present study, Experimental and controls were selected based on the detailed questionnaire. volunteers provided blood samples (5 ml) to establish cell cultures at 72 h. For karyotyping, 40 complete metaphase cells from each subject were evaluated. The detection of an increasing number of recognizable syndromes such as fragile X syndrome and Down syndrome were observed. Also major and minor Chromosomal aberrations such as deletions, inversion, gaps and breaks were found among the autism patients. The present study exhibits higher degree of chromosomal aberrations in experimental compared to controls ($P < 0.001$).

In conclusion, to point out that, the systematic study of autism is very rewarding with an accurate diagnosis in the proband and with proper

genetic counseling, one can alleviate the misery of many parent and also can reduce the birth of defected offsprings in the family subsequently. This study emphasizes the potential of analysing chromosomal rearrangements as a means to rapidly define candidate disease loci for further investigation.

P03.024 Carrier-fetus of balanced translocation t(5;20)(q34;p12)

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Translocations are considered the most frequent chromosomal changes in the human species. In genetics, a chromosome translocation is a chromosome abnormality caused by rearrangement of parts between chromosomes. Such balanced translocation are usually harmless to carriers and may be found through prenatal diagnosis. However, carriers of balanced reciprocal translocations have increased risk of creating gametes with unbalanced chromosome translocations leading to repeated miscarriages or delivery of chromosomal abnormal offspring. Genetic counseling and genetic testing is often offered to families that may carry a translocation.

Individuals who are carriers at balanced translocation usually appear completely normal and healthy but with reproductive problems. We found a carrier of balanced translocation t(5;20)(q34;p12) . We performed a cytogenetic analysis chromosomes of a fetus, after amniocentesis of 36 years old woman with history of 7 spontaneous abortions (each in the first trimester of pregnancy). Karyotype analysis revealed translocation between chromosomes 5 and 20. After cytogenetic study of both parents, mother was found to be a carrier of the same 5;20 translocation. The segments interchanged between chromosomes 5 and 20 were of almost the same size, and the arrangement of G-band was not significantly different.

Couples in which one partner is a carrier of a balanced translocation have an increased risk of infertility, repeated miscarriages and delivery of chromosomal abnormal offspring.

P03.025 Balanced and unbalanced forms of transmission of a complex chromosomal rearrangement involving chromosomes 3, 8 and 10 in two generations

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We report a familial apparently balanced complex chromosomal rearrangement (BCCR) involving chromosomes 3, 8 and 10. The proband is the fourth child from the eighth pregnancy of an unaffected mother whose karyotype was determined as 46,XX,t(8;10)(q13;q24),i ns(3;8)(q26.2;q22q23). Standard chromosome analysis of amniocytes revealed apparently the same balanced karyotype in the proband. However, at the age of three years the boy was referred for genetic analysis because of psychomotor delay, speech problems, autistic features, teeth irregularity and dysplastic ears. Array CGH and SNP array analysis were performed but yielded no clues, including no evidence for uniparental disomy. Proband's elder brother was born prematurely and died perinatally due to multiple severe malformations including bilateral cleft lip and palate, protruding premaxilla, right microtia with the absence of ear canal, deformation of legs, hypospadias and hypogenitalism. Chromosome analysis showed an unbalanced translocation t(8;10)(q13;q24) which led to partial monosomy of 8q. Proband's unaffected stepbrother has normal karyotype. Proband's unaffected stepsister has karyotype 46,XX,t(8;10)(q13;q24). In her case a recombination replaced the deletion in the translocated part of chromosome 8 with a segment of the normal chromosome 8. This, in combination with chromosome 3 lacking the insertion, led to a balanced karyotype with a simple reciprocal translocation. All findings were validated using FISH with chromosome paints. BCCRs are very rare in population. Our case illustrates multiple complex scenarios how the chromosomes affected by the rearrangements can be distributed to gametes and de-

scendants of BBCR carriers.

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P03.026 Deletion 3q26-28 in a patient with blepharophimosis-ptosis-epicanthus inversus syndrome (BPES)

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BPES is a rare genetic disorder with autosomal dominant pattern of inheritance. The frequency of BPES has been estimated to be 1:50,000. Patients with BPES have a combination of congenital anomalies as small palpebral fissures, epicanthus inversus, low nasal bridge and congenital ptosis. BPES has been categorized into two types: type I with infertility in females and type II involves eye malformation in both males and females. From the review of reported cases, it has been concluded that a locus for eyelid development is situated at the interface of long arm of chromosome 3. Since blepharophimosis, ptosis and microphthalmia are consistent features in Patients with BPES an interstitial deletion of band 3q2, the location of BPES gene at this position seems highly likely. Various reports linked the deletion in 3q21, 3q22, 3q23, 3q24, 3q25 and translocations t(3;7)(q26-pter: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. We report a sporadic case of BPES. We have done the Cytogenetic analysis of the patient. Standard GTG banding showed a novel deletion of band 3q26-28. This is the first report of deletion in this region. We also found deletion in the 3qter region which has been already linked to the BPES. This 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.

P03.027 Complex rearrangement involving 3 breakpoints in a t(3;22;11) translocation

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Complex chromosomal rearrangements (CCRs) are defined as structural chromosomal rearrangements with at least three breakpoints and exchange of genetic material between two or more chromosomes. We report a de novo complex chromosomal rearrangement involving chromosomes 3, 11, and 22 in a 9 year-old boy with mental retardation and dysmorphic traits (bushy eyebrows, frontal hirsutism, tented upper lip, downturned corner of the mouth). Using CGH microarray (Agilent®) and FISH controls, we evidenced a 21 Mb duplication of 3q, a 2.8Mb deletion in 11q, and 121 kb deletion in 22q. Chromosomal formula was

46,XY,der(22)t(3;22)(q26;q13),der(11)t(11;22)(q25;q13).
arr 3q26.3q29(177663316-199329792)x3,(11q25)(131617566-13443 2465)x1,(22q13.3)(43683481-43804487)x1 dn (reference : hg18).

The cryptic deletion on 22q breakpoint is underway by quantitative PCR. The parental karyotypes were normal. CCRs are extremely rare but often associated with mental retardation, congenital abnormalities and recurrent abortions. Dup(3q) syndrome is characterized by typical facial features, mental and growth retardation, often with congenital heart defects and a clinical overlap with Cornelia de Lange syndrome (CDLS). Del(11q) is known to be responsible of Jacobsen syndrome.

P03.028 Titin or a titin-like protein as a human centriolar protein ?

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Recently, the striated-muscle protein titin has been observed in non-muscle cells, and has been proposed to have a nuclear function as a chromosomal component. In this study, we further analyze this phenomenon. Different human cell-lines were used to characterize the immunolocalization of titin with different commercial titin-specific monoclonal antibodies: 9D10 (DSHB), 9B9 (Chemicon), 2Q1063 (USBiologicals), and T11(Abcam). These antibodies showed a varied location and expression characteristics of titin in the nucleus and cytoplasm but reacted not with condensed chromosomes. Here we report on the

development of a new mouse anti-titin monoclonal antibody, using the synthetic peptide corresponding to an amino acid sequence in the A-band of the titin molecule as immunogen. In the human skeletal muscle this MAb reveals a clear striated staining pattern, reacting with the A-band of the sarcomere. Electrophoretic, immunoblotting, and amino acid sequence analyses with ESI-MS/MS proved the target antigen of the MAb to be titin. The antibody reacts with titin also in non-muscle cells, giving a punctate pattern in cytoplasm and the nucleus. However, the most striking finding was a clear reaction also with centrioles in all cell types investigated so far. We suggest that this MAb detects titin or a titin-like protein in centrioles. The investigation of the association of the MAb target antigen in centrioles with other centriolar proteins is in progress. This work was partly supported by target financing SF 0188096s08 of the Estonian Ministry of Science and Education and Estonian Science Foundation (Grant No.6581).

P03.029 Combine analysis of chromosomal abnormalities in plutonium production workers: aneugenic and clastogenic aspects of the influence

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Combine analysis of clastogenic/aneugenic effects of plutonium production factors has been performed using conventional and interphase molecular cytogenetic methods. Frequencies of aberrations of routine stained and G-banded chromosomes, micronuclei and non-disjunction/lagging level in cytokinesis-blocked binucleated lymphocytes were estimated in lymphocytes of nuclear-chemical plant workers (66 males) and controls (25 males). The main contributing factors to increase of aberrations in plutonium workers were chromosome type aberrations (CTA), especially deletions (G-banding) and pair fragments (routine stain). Double-color FISH with centromero-specific DNA probes for chromosomes 2, 7, 8, 12, X and Y was used for the analysis of the chromosomal non-disjunction and lagging. The significant increase of the non-disjunction frequency has been found for all investigated chromosomes in the exposed group, except X-chromosome. The micronuclei (MN) frequency was also significantly higher in binucleated cells of nuclear industry workers ($p=0.009$). Correlations between markers of metaphase and interphase-FISH analyses have additionally been estimated. The results obtained by using different approaches have shown significant relationship. It figures that pair fragment frequency correlated with micronuclei level (Spearman rank (S_r) = 0.40). Moreover, CTA were connected with MN (S_r = 0.50) and non-disjunction (S_r = 0.45), deletions correlated with MN (S_r = 0.46), non-disjunction (S_r = 0.36), and lagging (S_r = 0.34). In general, the significant correlations have been found for basic characteristics of cytome abnormalities and different types of chromosomal aberrations. This observation points out that aneugenic and clastogenic events are related phenomena of plutonium mutagenic influence. This study was supported by Federal Agency of Education (P1707, P1748).

P03.030 Cytogenetic analysis in 550 couples with history of spontaneous abortions

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Chromosomal imbalance has been identified 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 in couples with history of spontaneous abortions can be made by conventional cytogenetics which involves karyotyping of GTG banded chromosome preparations obtained from whole blood. In the present study, conventional cytogenetic analysis was used to analyze peripheral blood samples from 550 couples with history of spontaneous abortions, mainly in the first trimester. Cytogenetic analysis revealed chromosomal abnormalities in 110 cases. Balanced reciprocal translocations were observed in 47 cases while Robertsonian translocations were observed in 6 cases. Novel cytogenetic anomalies like t(7;17), t(9;20) and t(17;20) 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=46) and 9 (n=50). Six cases revealed addition of genetic material in the short arm of chromosome 22. The detection of these chromosomal

anomalies, few of which are novel, in couples reiterates that cytogenetic analysis is a 'gold standard' for screening couples with history of spontaneous abortions where structural anomalies are observed more frequently than aneuploidies.

P03.031 Molecular studies on a supposed "satellited 8q" chromosome.

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One of the most common polymorphisms in a cytogenetic laboratory, is the presence of a satellite (NOR: nucleolus organizer regions) on a non-acrocentric chromosome. These extra NOR regions are easily confirmed by NOR-bands in the cytogenetic laboratory. They generally result from a translocation between a NOR region of an acrocentric chromosome and a non-acrocentric chromosome. These translocations are usually terminal and have been described in the literature on multiple chromosomes, the most frequent of which involving the Y chromosome (Yqs).

In most cases these polymorphisms are inherited without clinical repercussion, so when they are found in a karyotype usually any additional studies are not carried out. In fact, sometimes not even NOR-bands techniques are performed and the final diagnosis is done with only G-bands.

However, with the presentation of this case we want to show how important are such studies in order to properly define the "supposed satellited chromosome" and to exclude any other chromosome abnormalities.

Here we present a case of a 15-month-old girl who came to our laboratory with a karyotype in which a satellite in a chromosome 8 long arm (8qs) was described. The reason for the cytogenetic studies was a postnatal growth retardation, with all measures deeply below the 3rd percentile. In addition, the patient had mild dysmorphic features and slight motor delay.

FISH analysis with 8q subtelomeric probes (Cytocell) together with MLPA and Array-CGH analyses, showed a very complex 8q chromosome abnormality.

P03.032 Chromosomal radiation sensitivity in lymphocytes of breast cancer patients.

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Cytogenetic markers of intrinsic radiation sensitivity are seemed to predict genomic instability that correlates with the risk of cancerogenesis in healthy population and response to radiotherapy among cancer patients. Chromosomal G2-test makes it possible to estimate inter-individual difference in levels of DNA breaks and efficiency of DNA damage repair. Purpose of the presented study was comparative estimation of chromosomal radiosensitivity of lymphocytes in healthy donors and breast cancer patients.

Lymphocytes cultures were set up for 47 primary breast cancer patients and 44 healthy women. To examine chromosomal radiosensitivity cultures were irradiated with 0,5 Gy of X-rays on 48 hour (corresponds G2/M1). Metaphase preparations were made according to standard procedures. 100 well spread metaphases were analyzed for chromatid type aberrations.

It was shown that G2 phase is characterized by high chromosomal radiosensitivity and inter-individual variations in the levels of radiation induced chromosomal breaks in both groups of examined donors. Coefficient of variation in group of patients was 32% and 26% in controls. The mean overall G2 score for breast cancer patients was higher than that for the controls (86,6±10,4 and 67,8±14,0 respectively). Using the 90th percentile as the cut-off point, the proportion of breast cancer patients with high G2 sensitivity was 38 % compared to 12 % for the controls. There was no correlation between G2 sensitivity and spontaneous level of chromosome aberrations and age of donors. The results obtained support the concept of association between elevated chro-

mosomal radiosensitivity and genetic predisposition to breast cancer.

P03.033 Phenotypic Genotypic Correlation of Egyptian Patients with Chromosome 5 Aberrations

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Introduction: An increasing number of human diseases are recognized to result from structural variations - segments where DNA is deleted, duplicated or rearranged in which the clinical phenotype is a consequence of abnormal dosage of gene(s) located within the rearranged genomic fragments. Different chromosome 5 aberrations do occur including deletions, duplications, translocations, inversions and others. Aim: This study aimed to analyze the phenotypic features of different chromosome 5 aberrations and to correlate them with the type of aberration.

Subject and Methods: We were able to ascertain 12 cases with chromosome 5 aberrations. Cases included 6 children with mental subnormality and 6 couples seeking premarital genetic counseling and intrauterine fetal deaths. All the patients are subject to history taking, pedigree analysis, clinical examination and anthropometric measurements of the children. Chromosomal study of the couples, children and their parents was carried out using G-banding technique. FISH was performed using whole chromosome painting probes for confirmation.

Results: Our results showed different chromosomal 5 aberrations in the form of: del(5) (p14 - term), 5 p+, inv(5)(P13;q13), balanced translocations: t(3;5), t(5;7), t(5;11) and t(5;22).

Conclusion: Cases with larger deletions of chromosome 5 had greater degree of microcephaly. There may be a locus of Goldenhar syndrome on chromosome 5 extending from 5p14 to 5p15.2. The impact that these correlations have on understanding of copy number variations in the human genome will help clinicians not only for diagnosing genetic disorders in individuals with dysmorphism and developmental delay but also for prenatal diagnosis and genetic counseling.

P03.034 Chromosomes involvement in chromosomal structure rearrangements frequency in Lithuania

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Various congenital malformations, mental retardation as well as fertility problems could be determined by chromosomal structure rearrangements (CSR). Frequency of chromosomes participating in CSR does not correlate with chromosome length.

Assessment of the frequencies of the chromosomes involved in CSR in Lithuania patients referring for genetic counseling was carried out. Karyotypes of 2729 persons from Lithuanian population were analysed using conventional G-banded chromosome karyotyping, some CSR were verified with FISH and CGH methods. 106 chromosomal structure rearrangements (3.88% of all analysed cases) were detected. CSR were detected in chromosome X most frequently (8.5%), chromosomes 14 and 18 were most common autosomes with CSR, excluding Robertsonian translocations since only acrocentric chromosomes are involved into this type of CSR. No CSRs have been identified in chromosomes 19 and Y in the analysed group.

According to the origin of rearrangements formation detected rearrangements were classified into 9 groups. Reciprocal translocation was the most common type (29.25% of all detected cases) among balanced CSR while deletion dominated among unbalanced CSR (16.04%). Altered chromosome regions were located in chromosome regions with lower than genome average gene density, but that was not the only factor contributing to survival of foetuses with unbalanced CSR till birth. Pericentric inversion chromosome 9 has not been involved into the research as it is construed as heteromorphic chromosome variant. Only one more chromosome heteromorphic variant has been identified in the research - pericentric inversion chromosome 2 inv(2)(p11.2q12), although several different heteromorphisms are described in literature.

P03.035 A combination of molecular and conventional karyotyping to unravel a complex karyotype

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The use of high-resolution genome-wide array techniques as the first method of choice in the clinical diagnostic setting has been shown to considerably increase the diagnostic yield in patients with mental retardation with or without multiple congenital abnormalities. However, this technique is not able to elucidate more complex chromosomal rearrangements. We present a patient who was referred to the mental retardation clinic because of psychomotor retardation and non-specific behavioral problems. The 7-year old girl showed mild ptosis, retrognathia and strabismus and was therefore defined as only slightly dysmorphic. High-resolution genome-wide array analysis with a 250K SNP-array demonstrated four de novo interstitial deletions in three different chromosomes. Conventional karyotyping and FISH analyses revealed a complex chromosome rearrangement including both a reciprocal translocation and an insertion/deletion. This case demonstrates the necessity of using a combination of different techniques to unravel the nature of complex structural rearrangements.

P03.036 Cryptic genomic imbalances in two patients with *de novo* Complex Chromosome Rearrangements and abnormal phenotype.

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Introduction:

Complex chromosome rearrangements (CCRs) are structural chromosome anomalies involving more than 2 chromosomes or more than 2 breakpoints. Phenotypic abnormalities are found in about 50% of patients with *de novo* CCRs, suggesting that cryptic genetic imbalances, not detectable with conventional cytogenetics techniques, may be a common finding.

Here we report genomic imbalances detected in two patients with abnormal phenotype and CCRs using a high-resolution array for genome-wide DNA.

Patient's one conventional karyotype showed an apparently balanced translocation, t(4;8;7)(q13;q13;q11.2) dn. He presented with facial dysmorphism, psychomotor retardation and congenital malformations.

Patient two carried an apparently balanced translocation, t(3;5;16)(q25;q13.1;q13) dn. He also presented with facial dysmorphism, psychomotor retardation, congenital malformations and seizures.

Methods:

Array CGH experiments were carried out using Agilent Human Genome CGH Microarray (105K), with an average resolution of ~100Kb.

Results:

Array-CGH revealed cryptic genomic imbalances in both patients. Summarized results of all the aberrations are shown in table 1.

| Patient | Karyotype | Chromosome | Imbalance | Start (pb) NCBI Built 36 | End (pb) | Size (Mb) | OMIM genes |
|---------|---------------------------------|---------------------|-----------|--------------------------------|-----------|--------------|---------------------------|
| 1 | t(4;8;7) (q13;q13;q11.2) dn | 8q21.11- 8q21.13 | loss | 75079417 | 84816508 | 9.74 | GDAP1, PXMP3, ZFHX4 |
| | | 8q21.2-8q21.3 | loss | 86739116 | 88087692 | 1.35 | CNGB3 |
| | | 12q24.13 | loss | 112105455 | 112219269 | 0.11 | |
| 2 | t(3;5;16) (q25;q13.1;q13) dn | 5q15 | loss | 95457318 | 97818374 | 2.36 | PCSK1 |
| | | 5q23.2 | loss | 123137045 | 124391381 | 1.25 | |
| | | 5q33.3-5q34 | loss | 158629823 | 160676698 | 2.04 | IL12B |
| | | 5q34 | loss | 161228316 | 161537826 | 0.30 | GABRA1, GABRG2 |

Conclusion:

This study provides further evidence that cryptic genomic imbalances are common in patients with abnormal phenotype and "apparently

balanced" chromosome rearrangements. Furthermore, it reveals the complexity of chromosome rearrangements that is of great interest for the clinical diagnosis of these patients.

P03.037 Duplications 20p and 3q as a consequence of familial complex chromosomal rearrangement

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Balanced complex chromosome rearrangements (CCRs) are extremely rare. Their carriers have a high risk of primary infertility, spontaneous abortions or children with unbalanced karyotypes. We report an unusual case of a familial CCR involving three chromosomes.

An 8-year-old girl was referred for chromosome analysis because of heart defect, dysmorphic facial features, mild mental retardation and autistic features including a delay of speech development. Karyotyping revealed derivative chromosomes 20 and 22. For further clarification of the rearrangement, FISH was performed using 20p and 20q subtelomeric probes. It showed that material from 20p was translocated onto 22p. Array CGH was essential for the identification of the suspected third partner in this CCR: It showed a 3q duplication of about 16.5 Mb, and the CCR thus involved chromosomes 3, 20 and 22.

Karyotyping and FISH analysis of other relatives indicated that four members of this family are carriers of a balanced form of this CCR. These individuals are asymptomatic. Four of their descendants have two types of unbalanced CCRs. The proband and her maternal aunt have partial duplications of chromosome 3. Both have similar dysmorphic facial features and mental retardation, but the aunt is lacking the heart defect and autism. Two other individuals have partial duplications of chromosome 20. These children are currently very young. Their mental functioning seems to be normal, but the motor development is delayed and macrosomia is present in both cases.

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P03.038 Some cytochemical peculiarities of constitutive heterochromatin in cytotrophoblast chromosomes from human chorionic villi.

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Constitutive heterochromatin regions (CHRs) in chromosomes from chorion villi cytotrophoblast are characterized by decondensation, early replication, hypomethylation and DNase 1 hypersensitivity. After standard AO staining of untreated direct chromosomal preparations CHRs manifest unusually bright red fluorescence in 1qh, 9qh, 16qh, Yqh, 15cen, 22cen as typical for single-stranded DNA and RNA.

The nature of this fluorescence was studied on direct chromosomal preparations pretreated with different enzymes (RNase A, RNase H, DNase I, DNA ligase T4) followed by AO staining. CVS from legal and missed abortions at 5-10 week of pregnancy were used. Separate and combined pre-treatment of slides with RNase A and DNase I resulted in decrease of red fluorescence of CHRs in most metaphase plates. It was especially evident in 1qh. All chromosome arms were uniformly yellow-green or banded similar to RFA pattern in some metaphases. DNA ligase T4 or RNase H treatment resulted in total absence of fluorescence („black holes“) in CHRs, especially in 1qh, and uniform green fluorescence along chromosome arms. Staining patterns of chromosomes after treatment with corresponding buffer solutions only were identical to these one of untreated control slides.

These results could be attributed to conformational packaging of CHRs and thus might be treated as an evidence of obvious transcriptional activity of pericentromeric satDNA of 1qh in cytotrophoblast cells from chorionic villi during early embryogenesis.

P03.039 Cytochemical characteristics of constitutive heterochromatin regions from cytotrophoblast from human chorionic villi

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Although the biological significance of heterochromatin remained obscure for many years, it is now apparent that heterochromatin plays a number of roles in the organization and function of the genome. Numerous characteristics (especially proliferation and migration cells, invasive growth hormone-dependent, degree of methylation of DNA) cells of cytotrophoblast reveals a striking similarity to tumor cells therefore feasible functional active of constitutive heterochromatin regions (CHRs) of chromosomes 1, 9, 16 in cytotrophoblast cells of cytotrophoblast human chorionic villi is suggested.

The aim of investigation was comparative analysis of CHRs of chromosomes 1, 9, 16 stained by acridine orange of direct chromosomal preparations from cytotrophoblast human chorionic villi (from legal and missed abortions at 5-10 week of pregnancy) and peripheral blood lymphocytes from adult person.

As fluorescent dye AO is discriminated single-stranded nucleic acids from double stranded forms as orange-red and yellow-green fluorescence, respectively.

CHRs of chromosomes 1, 9, 16 after pre-treatment with RNase A revealed red fluorescence of undifferentiated cells cytotrophoblast, but these regions stained green in terminally differentiated culture lymphocytes. Absence of fluorescence in CHRs, especially 1gh, was observed after pre-treatment with RNase H.

These results confirm unusual conformation packaging of CHRs of 1, 9, 16 chromosomes in cytotrophoblast compared lymphocytes and might be treated as an evidence of obvious transcriptional activity DNA of CHRs of 1qh in cytotrophoblast cells from chorionic villi during early embryogenesis.

P03.040 Cytogenetic investigations in individuals residing near the Jagatjit industries at Hamira, Punjab, India

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Distillery effluents can affect the air, water and soil quality and hence, the ecosystem. Exposure from polluted surrounding near the Jagatjit Distillery may also be harmful to the residents of the area in terms of their genetic health and pose threat to their genetic make-up. In the present study, individuals residing near distillery unit were investigated for any chromosomal damage in their peripheral blood lymphocytes. The Cytokinesis block micronucleus assay was performed on 55 individuals: 20 males and 20 females residing near the distillery from birth/working in the distillery for 20-40y and 15 healthy controls matched for age (20-40 years), sex and socio-economic status. From each individual, 2000 binucleated (BN) cells were scored for the presence of micronuclei. Preliminary scoring has revealed percent frequency of micronucleated cells, nuclear buds and bridges to be higher in males in comparison to the values in females residing near/working in distillery indicating that these individuals have more chromosomal damage as compared to controls.

P03.041 Characterization by FISH and CGH-array of a partial monosomy 17 (p13.3, pter) and 21(q11.2q21.3) encompassing the 21q "critical region" in a patient presenting azoospermia and minor cerebral malformations.

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So far, only a small number of monosomy 21(q11.2-q21.3) cases have been reported. They displayed a very heterogeneous phenotype where most clinical features have been attributed to the deletion of the critical region 21q : APP- SOD1 resulting in the "arthrogriposis like syndrome". Here we report the case of a 26 years old patient referred for azoospermia. Clinical findings showed a moderate mental delay and facial dysmorphism. Cerebral MRI revealed an agenesis of the corpus callosum splenium and a cortico-sub-cortical atrophy. R banded

analysis revealed 17p13.3 monosomy associated to a 21 (pter,q21) monosomy as a result of unbalanced segregation of a reciprocal maternal translocation (45, XY,der17 t(17; 21)mat,-der 21). FISH studies on the proposita lymphocytes confirmed the terminal deletion 17p and the deletion of the 21(pter,q21) region. CGH-array analysis estimated the size of the 17p telomere loss to 681 kb and the deletion of the chromosome 21q to 16,15Mb. The study showed that the breakpoint in chromosome 21 is proximal to the gene APP, the proximal boundary of the "monosomy 21" critical region and the breakpoint in chromosome 17p is telomeric to LIS1 gene. Azoospermia could be explained by the unbalanced translocation since that this chromosome anomaly is known to block meiosis during spermatogenesis. On the other hand, it is often reported, in 17p terminal deletion cases with retention of gene LIS1, a severe mental delay in association with hypotonia and cerebral malformations suggesting the involvement of other genes other than LIS1 in brain development and neuronal migration.

P03.042 Interstitial deletion 11q13.1-q13.3 in a boy with developmental delay, prenatal short stature, facial dysmorphisms and malformations of the hands and feet

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We report on a 3 year-old boy with mental retardation, prenatal short stature, microcephaly, split hand and facial dysmorphisms in association with an interstitial 11q13 microdeletion. On the patient's left hand, the second finger was missing, there was total osseous syndactyly of the phalanges III/IV and flexion contraction in the metacarpo-phalangeal joint of the thumb. On the right hand, the distal phalanx of the second finger was hypoplastic with missing nail, there were complete cutaneous syndactyly III/IV and a finger-like thumb. There was syndactyly I/II and III/IV on the right foot. On the left foot there was shortening of the third toe, and lateral deviation of the distal phalanx of the second toe but no syndactyly. The babygram showed 11 ribs and a rather plump sacrum. Brain ultrasound revealed a single plexus choroideus cyst.

HR-CGH showed an interstitial deletion of 11q13. Array-based CGH (44K array, Agilent Technologies) confirmed the deletion and revealed a proximal breakpoint between positions 66.58 Mb and 66.64 Mb and a distal breakpoint between 71.43 Mb and 71.46 Mb, thus a deletion size of 4.73 Mb [46,XY,del(11)(q13.1q13.3)].

This is the first report about a patient with this particular aberration. There are interesting candidate genes within the deleted region: such as TBX10 (malformation of the ears, mental retardation, missing rib and plump sacrum), FGF19 (malformation of the ears, mental retardation, sparse subcutaneous adipous tissue) FGF3 (malformation of the ears, microdontia) FGF4 (malformation of the limbs), SHANK2 (mental retardation) and others associated with eye-disease and deafness (CABP4, TCIRG1, LRP5).

P03.043 Interstitial chromosome 6q23 deletion characterized by CGH in a patient with two *de novo* apparently balanced translocations, t(6;10) and t(7;11), and a paternal t(2;13)

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We report on a female with a rare complex chromosomal abnormality involving three translocations, which were characterized by GTG banding: two *de novo* apparently balanced t(6;10) and t(7;11) translocations, and a paternal t(2;13). To define the three translocations breakpoints and possible imbalances, comparative genomic hybridization (CGH) techniques were now used, which allowed us to detect an interstitial 6q23.1-6q23.3 deletion.

The genetic investigation of this patient started when she was 14. Her clinical features are: mild to moderate mental retardation (the neuropsychological profile showed global IQ of 58), dysmorphic facial features with microcephaly, strabismus and microtalmia, sensorineuro-deafness, recurrent sinopulmonary infections, immunodeficiency and hemolytic anaemia. The diagnosis of Nijmegen Breakage Syndrome (NBS) was suspected and both cytogenetics (chromosome breaks) and molecular (nibrin gene) studies were performed 11 years ago, but these results were negative. Despite these results, we cannot exclude this clinical diagnosis. The patient is currently 27-year-old and

has been doing follow-up studies regarding her imuno-hematological problems. The authors enhance the importance of using molecular cytogenetic techniques for detecting subtle chromosome imbalances in patients with atypical phenotypic characteristics, especially with complex *de novo* rearrangements and compare the present case findings with previously published similar data.

P03.044 Clinical findings of 32 individuals with deletions of 18p

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One of the primary goals of the Chromosome 18 Clinical Research Center is to identify genotype-phenotype correlations. An important part of this process is an extensive and accurate phenotypic description. As a part of this process, we are conducting a thorough review of the phenotype of individuals with 18p-. Here, we report the first part of this project: the phenotypic characterization of study patients with a centromeric breakpoint. In addition to reviewing the medical records of 32 study participants, we have interviewed the parents of 28 of these individuals. Neonatal complications (jaundice, feeding problems, or respiratory distress) were present in 20 individuals. Hypertonia or hypotonia were reported in 28 participants. Holoprosencephaly or findings frequently associated with holoprosencephaly (such as single central incisor or caudal regression) were identified in nine patients. Four had seizure disorders. Hearing and vision problems were common, occurring in seven and 24 patients respectively. Eighteen patients had chronic otitis media. Of the thirteen that had undergone echocardiograms, eight had a heart anomaly. Gastrointestinal anomalies were fairly common. Ten had chronic constipation; five had reflux. Hernias were present in six patients, and volvulus in one patient. Six had scoliosis; five had pectus excavatum. Foot anomalies were present in six individuals. Endocrine problems were also fairly common. Two had panhypopituitarism, while seven had isolated growth hormone deficiency. Four patients had hypothyroidism. As we continue to collect phenotypic data on patients with non-centromeric breakpoints, we hope to narrow critical regions for features of 18p deletions.

P03.045 A new case of deletion 18p syndrome

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Deletion of the short arm of chromosome 18 is a well established chromosomal aberration. In patients, there is a broad variability of phenotypic features. Facial dysmorphism, short stature, skeletal anomalies, and more or less severe psychomotor retardation are commonly seen. We report a patient with a del 18p, who was referred to our department. Our propositus is 12 years old and the last child born to healthy, non-consanguineous parents. At birth, his father and his mother were 43 and 42 years old, respectively. He has two sisters and one brother, all are healthy. He was delivered full-term with a normal delivery, and a birth weight of 3150 g. He had developmental delay, in all aspects specially walking. He lost all his child teeth by the age of 3, due to poor quality and extended caries.

His phenotypic manifestations are flattened nasal bridge, unilateral strabismus, large mouth, broad philtrum, low-set and rotated ears, very large and detached pinnae, and brittle nails.

His mental status is in agreement with moderate mental retardation, and according to Weschler intelligence scale for children, his IQ was 77. He performed in non-verbal functions better than verbal ones. He showed delayed in learning at school, abnormal behaviour, agitation and aggressivity. He has seizures and his thyroid hormones showed high level of TSH.

Cytogenetic studies from peripheral blood lymphocytes was carried out using G-banding technique on the propositus and his parents.

The karyotype of the propositus revealed a *de novo* of 46,XY,del(18p)(p11.3) and his parents showed normal karyotypes.

P03.046 Report of a patient with a 46, XY, 9p- constitution due to a paternal t(5;9) translocation

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Background. Deletion 9p syndrome is a rare congenital syndrome. It is characterized by mental retardation, trigonocephaly and other dysmorphic features. We report of a patient with a 46, XY, del(9p) constitution

due to a paternal translocation t(5;9), diagnosed in Lithuania.

Case report. The parents of the proband had two healthy children from their first marriages. This boy was delivered at 32 weeks of gestation by a caesarean section due complicated pregnancy, breech presentation. At birth his weight was 1980g, length 44cm and head circumference 27cm. Clinical examination revealed trigonocephaly, upslanting palpebral fissures, epicanthus, hypertelorism, flat nasal ridge, anteverted nostrils, long philtrum, cleft palate, micrognathia, low set ears, widely spaced nipples, corrected hernia umbilicalis, micropenis, partly descended testis, underdeveloped scrotum. A large hemangioma on the abdomen and occiput regions was observed. Cardiac evaluation revealed a ventricular septal defect. Gastrostoma was performed due to swallow difficulties. The chromosome analysis of peripheral blood lymphocytes revealed 46,XY, del(9p) (p22.3) karyotype. Cytogenetic analysis was performed from cultivated peripheral blood lymphocytes, the resolution level was over 550 bands. Karyotype analysis of both parents revealed normal mother karyotype and a balanced t(5;9)(p15.3;p22.3) translocation in the father, which has been confirmed using subtelomeric FISH analysis.

Conclusion. The present case illustrates that balanced translocation carrier parents have high probability of having baby with 9p- syndrome.

P03.047 Supernumerary minute chromosome 17 in a boy with severe developmental delay: molecular breakpoint in the unstable proximal 17p region

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Introduction: Small supernumerary marker chromosomes (sSMCs) are structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional cytogenetics alone. Particularly, chromosome imbalances involving the short arm of chromosome 17 result in various distinct clinical conditions.

Clinical Report: We describe a 5 years 6 months old boy with microcephaly, mild dysmorphic features and severe global developmental delay.

Methods: Chromosome analysis was performed using conventional GTG-banding techniques. To identify the origin of the *de novo* sSMC, FISH analysis was performed using centromere-specific multicolor FISH. Single Nucleotide Polymorphism (SNP) Oligonucleotide Microarray Analysis was also carried out.

Results: A very small supernumerary marker chromosome was identified in 85% of blood lymphocytes and FISH studies showed that the marker was derived from chromosome 17. Several unique sequence probes mapping to the SMS (17p11.2) and CMT1A (17p12) critical regions were applied to assign the distal breakpoint on the der(17) chromosome, which was mapped at 17p12 adjacent to a low-copy repeat (LCR) sequence, while the proximal breakpoint mapped within the centromere. The karyotype was 47,XY,+mar[51]/46,XY[9].ish der(17).

Conclusions: This represent the first case of a marker chromosome 17 to be characterized at the molecular level. It is important that more cases of sSMC are described using high resolution molecular techniques in order to elucidate a detailed genotype-phenotype correlation. Furthermore, a precise determination of the chromosomal breakpoints will enable the description of the mechanisms of origin of recurrent chromosomal rearrangements like the genomic disorders of the unstable proximal 17p region.

P03.048 DiGeorge critical region 2 deletion in a patient with delayed psychomotor development and bilateral sensorineural hearing loss

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Partial monosomy 10p is a rare chromosomal aberration (approximately 50 cases in the literature); it is considered a contiguous gene syndrome. Two distinct critical regions have been described, one named HDR1, responsible for hypoparathyroidism, sensorineural deafness, and renal anomalies (HDR-syndrome) and one named DiGeorge critical region 2 (DGCR2), associated with congenital heart defects and thymus hypoplasia/aplasia or T cell defect, respectively.

We report the case of a 4-year-old boy presenting with facial dysmorphisms, delayed psychomotor development and bilateral sensorineural hearing loss with an interstitial deletion of chromosome 10 short arm (10p13-10p14), the smallest deletion found in the literature so far.

Our patient, carrying a deletion of the entire DGCR2 region and a partial deletion of the HDR1 region, showed, unexpectedly, only few minor clinical features of DiGeorge 2 syndrome (psychomotor retardation, palpebral ptosis, epicanthic folds, anteverted nares, cryptorchidism, hand/foot abnormalities) and did not show typical signs, such as cardiac defect, cleft palate and reduced T cell population. Furthermore he had sensorineural deafness which is one of the characteristic features of the HDR syndrome. Our data support the hypothesis that DGS2 syndrome is associated to locus heterogeneity. We hypothesized that the genes responsible for deafness in HDR syndrome or regulatory elements of these genes map in the region between the two critical regions, namely HDR1 and DGCR2 (Lichtner et al., J Med Genet 2000).

P03.049 Duplication 8q12: a recognisable phenotype with Duane anomaly.

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Duane anomaly is a frequent ophthalmologic feature observed in several genetic disorders such as Oklahiro syndrome (acro-renal-ocular syndrome) or Wildervanck syndrome. Here we report on a patient with a small duplication of the long arm of the chromosome 8 (8q12, found with SNP-array) presenting with duane anomaly, moderate mental retardation and facial features namely horizontal eyebrows with proximal flaring of eyebrows, long palpebral fissures, and full cheek and lips. We found two other patients in the literature with a 8q12 duplication and a similar phenotype. The minimum critical size of the 8q12 duplication was approximately 1.2 Mb and encompassed 4 genes namely the *CA8*, *RAB2*, *RLBP1L1*, and *CHD7* genes.

The observation of a duplication of the these genes questioned us on the potential effects of gene dosage in the phenotype observed in patients with 8q12 duplication. To our knowledge, no information is available in the literature regarding pathological effects secondary to overexpression of the *CA8*, *RAB2*, *RLBP1L1*, or *CHD7* genes. However, *CA8* loss of function mutations have been recently demonstrated to be responsible for ataxia and mild mental retardation, the *RAB2* gene is involved in neuronal adhesion, and neurite growth *in vitro* and hemizygous loss of function of the *CHD7* gene lead to CHARGE syndrome suggesting a role of these genes in the phenotype observed in 8q12 duplicated patients. We believed that patients with 8q12 duplication share a common recognizable phenotype and we propose that 8q12 duplication should be considered in patients presenting with a syndromic form of Duane anomaly.

P03.050 Results from CEQA 2009, the European Cytogenetic External Quality Assessment Scheme

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CEQA undertakes annual external assessment of laboratory performance by provision of cytogenetic images and case details for analysis through its online website. For 2009, two cases each of postnatal blood, prenatal amniotic fluid, haemato-oncology, and pre-implantation blastomeres were given, with 71, 72, 46, and 30 laboratories respectively submitting returns. Also, a new pilot EQA scheme for array-CGH

using distribution of a DNA sample, open to 30 participants, was run in collaboration with the European Molecular Genetics Quality Network (EMQN).

The poster outlines details of the cases provided, including images and exemplary reports. The marking criteria are explained, whereby the specialist groups of assessors assigned a score for each submission based on the criteria of analytical accuracy, interpretation of the significance of the result, and content of the report. Results from all the EQAs are summarised and examples of "Critical Error" are included, defined as an error that would result in inappropriate clinical intervention. Such errors were found in 27 of the 438 (6%) overall case submissions for the established (non-pilot) schemes.

P03.051 Fanconi anemia: Telomere and Telomerase genes evaluation using FISH technique.

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Introduction: Fanconi anemia (FA) an autosomal recessive and X-linked disease which belongs to the group of chromosome instability with high risk of malignancies. Because telomeres are involved in chromosomal stability and cell proliferation, telomeric shortening and deletion will accelerate cell apoptosis and the development of anemia.

Aim: The study aimed to assess the telomeric deletion and the telomerase genes (TERC and TERT) copy number in patients with FA and aplastic anemia, compared to controls.

Subjects and methods: The study included 10 patients with aplastic anemia (7 FA and 3 non FA) and 5 healthy individuals (control group). DEB, All Human Telomeres, TER, and TERT Gene FISH probes, were applied for peripheral blood lymphocytes culture.

Results: There was statistically significant difference ($p<0.05$; unpaired Student's t-test) in the telomeric deletion of FA patients in relation to controls ($P< 0.0003$), and a significant telomeric deletion in aplastic anemia patients (non FA) in relation to controls ($P<0.016$), but no significant difference in the deletion between FA and non FA ($P< 0.16$). The extra-telomeric signals (intrachromosomal and extrachromosomal) were increased in FA and non FA patients. No amplification was detected for telomerase genes in all patients.

Conclusion: Marked increase in the deletion of telomeric ends was detected in all patients. Although in the literature telomerase enzyme reactivation was reported, no amplification of telomerase genes were detected in our patients. Therefore we suggest that the increase in the telomerase enzyme level can be due to other factors than gene amplification.

P03.052 Chromosomal alterations and sister chromatid exchange due to fluoride effects in and around the Dharmapuri districts of Tamil Nadu

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Fluorides are organic and inorganic compounds containing the element fluorine. Fluorides are generally colorless and they are more or less soluble in water and can take the form of a solid, liquid or gas. Fluorides are important industrial chemicals with a number of uses but the largest uses are for aluminum production, drinking water fluoridation, and the manufacture of fluoridated dental preparations. Water with underground sources is more likely to have higher levels of fluoride. Hence the present investigation has been carried out in the Dharmapuri districts to analyse the fluoride effects due to water. The objective of the present study is to investigate the cytogenetic effects due to fluorides among the population in Dharmapuri district, South India. In the present study totally 50 samples 25 experimental and 25 controls were selected. All the experimental were subjected to chromosomal alterations (CA), Sister Chromatid Exchange (SCE) and Micronucleus (MN). Higher degree of CA like deletions, translocations, dicentrics, chromatid gaps, breaks and satellite formation were observed. SCE and fewer MN formation were also observed in experimental when compared to their respective controls. In conclusion, the people residing in the Dharmapuri district were found to have a higher level of genetic damage and the fluoride effect is hall marked by specific behaviors of gene environment relationship which probably defines the cellular pathobiology of these fluorides diseases.

P03.053 Analysis of factors associated with the CGG expansion of the FMR1 gene in a Basque sample: Use of Single Nucleotide Polymorphisms (SNPs)

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The expansion of a CGG trinucleotide repeat in the 5'untranslated region in the first exon of the FMR1 gene is the main cause of the fragile X syndrome. This gene maps to Xq27.3 and coincides with the fragile site FRAXA. There are some important factors that influence the instability of the repeat. These factors include the length of the repeat, the AGG interspersion pattern and the DXS548-FRAXAC1 microsatellite markers associated haplotypes. It has been suggested that the use of more stable polymorphisms, like Single Nucleotide Polymorphisms (SNPs) could provide new advances in the study of the CGG repeat instability. In previous investigations based on the structure of the CGG repeat and linked microsatellite markers we showed differences among the Basque populations analyzed. In the present report, in order to complete the investigation about the CGG repeat instability, we have studied seven SNPs in the vicinity of the FRAXA repeat (DXS548P, WEX28, WEX1, ATL1, FMRb, WEX17 and WEX10) in Markina and Arratia, two isolated Basque Valleys of the Biscay province. Our preliminary results suggest that there is a preferential association between DXS548-FRAXAC1 haplotypes and the SNPs ATL1*G, FMRb*A and WEX28*G in both valleys. On the other hand, our data indicate that several of the analyzed SNPs predispose to the instability of the CGG repeat.

P03.054 Cytogenetic techniques in management of haematological malignancies: Degenerating or gold standard?

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Conventional cytogenetic techniques played an important role in diagnosis and monitoring of CML by characterizing Philadelphia chromosome, which has heralded the development of gleevac. Subsequent development of FISH technique has enabled faster and sensitive detection of onco-/anti-oncogenes. Nevertheless introduction of PCR and microarray techniques has revolutionised the management of haematologic malignancies and development of molecular and individualized medicines. PCR based studies require stringent lab-environment and validation of quantification of transcript ratio failing which a false positive result may create confusion among clinicians and patients. Large scale clinical trials are being conducted for standardizing real time PCR techniques for monitoring MRD condition.

FISH technique, though generate false positive signals and is specific, is reliable and getting refined with advancement of probe-design and combinatorial application with simultaneous detection of surface antigens (FICTION). Clonal evolution or multilineage condition can only be detected by conventional G-banding technique and molecular screening. The whole genome screening, though possible by SKY or CGH techniques, requires validation and refinement in disease management. Limitation of resolution of conventional cytogenetic technique many times reproduces a normal karyotype and that indicates the need for employment of PCR technique especially for JAK2, NPM1, etc. Therefore, it is clear that every technology has some merits and demerits where G-banding technique is still being considered as primary step for diagnosis and relapse-evaluation. In known cases, FISH could be a better choice. Thus, with the present day technologies available, conventional cytogenetic technique cannot be replaced and be considered as the gold standard in clinical management.

P03.055 Complex glycerol kinase deficiency in a patient with psychomotor retardation: molecular analysis and comparison to published data.

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Glycerol kinase deficiency (GKD) is a rare X-linked recessive disorder occurring in two forms, isolated and complex. Isolated GKD is caused by point mutations in the glycerol kinase gene (GK). Complex GKD is a contiguous gene syndrome involving the GK locus together with other Xp21 sequences including e.g. the adrenal hypoplasia congenita (NR0B1 gene) and/or the Duchenne muscular dystrophy (DMD) loci. We present an infant hospitalized at the age of 6 weeks with ionic imbalance, renal failure, and elevated creatin kinase and liver markers. The child suffered from hyponatremia, dehydratation, proteinuria, high level of glycerol in urine and weak spontaneous mobility. Today, at the age of 2 years, he also shows mild psychomotor retardation.

MLPA analysis of the DMD gene revealed a deletion of all exons. PCR analysis confirmed a deletion of GK, NR0B1 and IL1RAPL1 genes. Karyotype analysis showed 46,XY,del(X)(p21.2p21.3), which was also confirmed using FISH. These analyses confirmed suspected diagnosis of complex GKD. Array CGH analysis showed an 8.7 Mb deletion containing approximately 20 protein-coding genes. The distal breakpoint was located between DCAF8L1 and IL1RAPL1, and the proximal one was located in the PRRG1 gene.

About 100 patients with complex GKD and phenotypes reflecting the variable size of their deletions have been reported. Characterisation of the deletions is therefore an important prognostic factor. Our patient carried a rather large deletion including also the IL1RAPL1 gene associated with X-linked mental retardation and autism.

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P03.056 Comparative analysis of acetylated histone H3 and methylated DNA distribution along human lymphocyte chromosomes

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At the first step of our research we have studied how chromatin regions, enriched in acetylated H3 (AcH3K9), are distributed along human metaphase chromosomes. Metaphase chromosomes were obtained from PHA-stimulated human lymphocytes, fixed with 2% glacial acetic acid in ethanol. 60 metaphases (~400 bands per haploid genome) from 5 individuals were studied. Analysis with monoclonal antibodies against AcH3K9 (Abcam, USA) revealed clearly distinguishable regions with high, medium, weak or absent fluorescence on metaphase chromosomes. Combined DAPI and immunofluorescent staining allowed co-localization of the regions enriched/depleted in AcH3K9 with Q-negative/positive bands (R/G-bands). AcH3K9-richest regions corresponded to 32 R-bands. Medium to weak AcH3K9-signals were detected in 86 R-bands. All G-bands demonstrated weak fluorescence. In heterochromatic blocks of chromosomes 1, 9, 16 and Y immunofluorescent signal was absent indicating no acetylation of H3K9 in these regions.

At the second step we have compared H3K9 acetylation with DNA methylation status, described in our previous study (Pendina et al., 2005). Non-acetylated heterochromatic regions were hypermethylated. Among R-bands with medium to weak acetylation 6 bands demonstrated weak, 48 - medium and 32 - high DNA methylation level. 32 AcH3K9-richest R-bands showed variable DNA methylation level: from high in 17 bands and medium in 12 bands to low in 3 bands - 1p13, 2q31, 2q33. Low level of DNA methylation in highly acetylated bands favors their specific function in lymphocytes.

Thus, the co-location of acetylated and methylated chromatin regions suggests that active and inactivated chromatin is band-specifically distributed along chromosome arms.

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P03.057 Identification of recurrent cytogenetic aberrations in hepatoma patients in southern India

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Hepatoma (Primary liver cancer) is the cancer of the hepatocytes. It usually grows in the liver as a ball-like tumor, invading the normal tis-

sue surrounding it. The aim of the present study was to identify the Chromosomal aberrations in hepatoma patients to assess whether peripheral blood had non-random cytogenetic aberrations as observed in tumor samples. The study was conducted on the peripheral blood of 67 hepatoma patients (aged 30 to 85 years male) undergoing hepatic resection of liver tumour with curative intent. In the present study all the experimentals and controls were analysed chromosomal alterations using conventional G-banding.

In the present investigation cancer patients had significantly increased aberrant metaphases compared to controls. Hepatoma samples revealed frequent aberrations in 1q, 8q, 17q, 20q, 4q, 8p, 13q, and 16q. Our finding of a high incidence of 1q gain and frequent deletion in the short arm of chromosome 8 strongly suggested this aberration was associated with the development of this disease. Chromatid breaks were seen on chromosomes 1p, 2p, 2q and 4q while chromatid gaps were on chromosomes 1p, 2p, 3p, and 3q.

The results of this study might help in providing important clues and to add better knowledge in the location of relevant genes on specific altered regions of chromosomes. Comprehensive elucidation of the specific genes and molecular pathways involved in progression from pre neoplastic lesion to frank neoplastic in the protracted process of hepatocarcinogenesis will facilitate development of new strategies for prevention and therapy.

P03.058 New chromosome aberrations in male hypogonadism

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Hypogonadism is a lack of testosterone in male patients and can be of central (hypothalamic or pituitary) or testicular origin. The molecular basis of hypogonadism has been identified for only 25%-30% of patients, with mutations mostly in the KAL1, FGFR1 and GnRHR genes. Although the systematic chromosome study of patients is a powerful approach in the search for new loci associated with diseases, there is little data, other than isolated case reports, documenting the karyotype of a more larger sample of hypogonadotropic patients.

In this context we have studied cytogenetically 51 patients with primary hypogonadism (n=28) and secondary hypogonadism (n=23) classified by the gonadotropins level. Chromosome analyses were performed from peripheral blood lymphocyte cultures using GTG, C-banding and fluorescence in situ hybridization (FISH) methods. Of 51 patients studied, 34 were normal. However, 17 males (33.3%) had major chromosome abnormalities with sex chromosome abnormalities in 14 (27.5%) cases and autosome abnormalities in 3 (5.9%) patients. All three males were with secondary hypogonadism. They had chromosome abnormalities, previously not described in the patients with secondary hypogonadism, such as supernumerary inv dup(22)(q11.1); 45,XY,t(13;14)(q10;q10), and 46,XY,t(5;13), all confirmed by FISH method.

The revealed new cases, particularly translocations with new break-events provide valuable information for positional cloning of new secondary hypogonadism genes, which might be involved in the pathogenesis of male secondary hypogonadism. Supported partly by target financing SF 0180096s08 of the Estonian Ministry of Science and Education.

P03.059 X chromosome monosomy restricted to the left ventricle is not a major cause of isolated hypoplastic left heart syndrome (HLHS)

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Hypoplastic left heart syndrome (HLHS) has an incidence of 0.016-0.036% live births. HLHS encompasses a spectrum of heart anomalies characterized by obstructive lesions of the left side of the heart accompanied by varying degrees of underdevelopment of the aorta, aortic valve, left ventricle, mitral valve, as well as mitral atresia or stenosis. The aetiology of HLHS is heterogeneous and can be observed as an isolated defect or in association with different chromosome abnormalities. In particular, HLHS has been reported in about 20% of patients with 45,X karyotype, suggesting that monosomy X is a cause of this developmental heart defect.

The aim of this study was to evaluate if X chromosome monosomy restricted to the left ventricle was associated to isolated HLHS. Formalin-fixed, paraffin-embedded cardiac tissue of 19 patients, 10 males and 9 females, affected by HLHS without extracardiac congenital anomalies and with a euploid karyotype, were investigated. For each sample 50 consecutive nuclei isolated from both ventricles were analysed in a blind fashion by interphase FISH with X/Y/18 centromeric probes. The same analysis was performed on a group of 15 chromosomally normal pediatric patients affected by either restrictive or dilated cardiomyopathy, as negative controls. Mosaic monosomy X was detected in the cardiac tissue nuclei of both groups, with similar frequencies (6-16% and 12-16%, respectively). In conclusion, this study has excluded that X chromosome monosomy confined to the left heart is a major cause of HLHS, arguing that different pathogenic mechanisms are responsible for syndromic and isolated HLHS.

P03.060 Prenatal detection and follow-up of a mosaic i(18p) case. A complex case for genetic counseling.

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Prenatally detected mosaic aneuploidy leads to problems in genetic counseling. The risk for an abnormal clinical outcome varies according to the chromosomes and tissues involved and to the percentage of cells with unbalanced karyotype. We present a prenatal finding of mosaic tetrasomy 18p detected at amniocentesis, not confirmed after birth in cord blood and skin, but present in placental tissue, buccal smear and cells from urine of the baby.

Case report: Amniocentesis was done to 42-year-old woman due to positive serum-screening for Down syndrome, normal ultrasound. Two cultures were set up, chromosomes analysed by G-banding and FISH. Unbalanced mosaic karyotype with tetrasomy 18p in 17% of cells was found: 46,XY[47]/47,XY,+i(18)(p10)[18].ish i(18)(p10)(pter+,D18Z1+,pter+).

Parents opted to continue pregnancy. Apparently normal male baby was delivered prematurely (31wp, preeclampsia).

Postnatal chromosomal analysis of cord blood (22 mitoses, 70 nuclei analysed) and skin fibroblasts (17 mitoses, 100 nuclei) showed normal male karyotype. Unbalanced mosaic karyotype +i(18p) was confirmed in ~20% of cells in placental tissue (35 metaphases, 55 nuclei) thus suggesting to the diagnosis of confined placental mosaicism.

Subsequent FISH analysis of buccal smear (100 nuclei) and urine sediment cells (50 nuclei) confirmed the presence of +i(18p) in mosaic form in the tissues of baby as well (17% and 24% respectively). In this case the final diagnosis of true mosaicism (involvement of entodermal and ectodermal tissues) was made thus giving the prognosis of facing developmental problems in near future.

Clinical data of our patient at birth and at 7 months of age will be presented.

P03.061 A liability to malignancy, immunodeficiency, facial abnormalities and chromosomal instability without mutation in DNMT3B gene in two siblings: new chromatin disorder delineation?

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Several human diseases which are characterized by alterations or modification of chromatin structure caused by changes of methylation pattern are considered as so-called 'chromatin disorders'. ICF syndrome (OMIM #242860) belongs to the aforementioned disorders and is a very rare recessive disease caused by mutations of the gene *DNMT3B* that encodes the 'de novo DNA-methyltransferase 3B'. We report on a family with ICF-like symptoms but without *DNMT3B* mutation and with a rather atypical pattern of heterochromatin abnormalities.

Methods. Chromosomal stability of both sisters and their parents using GTG-banded or standard solid-stained chromosomes of 48, 72 and 96 h blood lymphocyte cultures and karyotypes investigation performed on the base of conventional cytogenetics. Molecular cytogenetics

approaches were performed for further investigation of specificity of cytogenetic instability in this family. Two-color interphase FISH using centromeric probes for X/Y-chromosomes (Abbott/Vysis) and multiplex-fluorescence in situ hybridization (M-FISH) analysis was applied to clarify the composition of complex aberrations, irresolvable by conventional methods. The analysis of chromosomes most frequently involved in aberrations was performed. 100 metaphases were analyzed per individual. Epstein Barr virus based immortalization of the family blood was done.

Results. Karyotypes of both sibs and their parents were normal. Evident specificity of chromosome instability was presented by high frequency of juxtacentromeric heterochromatin rearrangements, chromosomal fragments and despiralization or pulverization. However, mutations in *DNMT3B* could not be detected.

Conclusion. Clinical, molecular genetic and cytogenetic findings will be presented. The discovery of a new so-called 'chromatin disorder' is suggested.

P03.062 The results of cytogenetic analyses in 407 infertile women in Prešov region, Slovakia

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The aim of this study was to assess the frequency of chromosome anomalies in infertile women from Prešov region (Slovakia) in 1998-2009. We analyzed the karyotypes of 407 individuals with clinical diagnosis of infertility included primary sterility, secondary sterility and repeated abortions using cytogenetic banding techniques. We detected fifteen aberrant karyotypes that corresponds to chromosome aberrations frequency of 3.7%. Chromosomal polymorphism was detected in 17.7% of individuals. The most frequent heterochromatin variant detected in 4.7% of women of study survey was 15s+. Increased length of heterochromatin and satellites in autosomal chromosomes were present in 9.2% of women. The results of our study confirmed the higher occurrence of chromosome anomalies in infertile women that absolutely reasons indication of cytogenetic examination.

P03.063 Chromosomal instability in peripheral blood lymphocytes and risk of inflammatory bowel disease (IBD) in south Indian population

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Inflammatory Bowel Disease (IBD) is a chronic idiopathic inflammation of gastrointestinal tract. The two main forms of IBD are Crohn's Disease (CD) and ulcerative colitis (UC). In the present study about 43 patients were investigated and they were analysed for different chromosomal alterations using standard protocol. Hence blood samples were collected from various hospitals of Coimbatore district after obtaining the consent of patients. Then the patients were divided into four groups along with suitable age matched normal healthy controls for each group. In the present study, all the patients and controls were subjected to chromosomal analysis using the standard protocol. 14 of the 43 (32.5%) patients and 2 controls of the 43 (4.65%) were found to have different chromosomal aberrations. Group I patients were found to have the maximum number of chromosomal aberrations with 8 patients (18.6%) of the 43 patients displaying aberrations like deletion, translocations, mosaic and satellite. In group II, four patients (9.3%) were found to have different chromosomal aberrations like translocation and deletion. In group III, two patients (4.6%) were found to have aberration of deletion type. None of the group IV patients displayed chromosomal aberrations of any type. Two of the controls in group I showed minor aberrations (4.65%). This study in its own small way might contribute valuable nuggets that could aid in solving the complex clues underlying this multifactorial disease and which are on the rise in Indian population.

P03.064** Parental insertional balanced translocations are an important cause of apparently *de novo* CNVs in patients with developmental anomalies.

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Array CGH screening is becoming a first tier diagnostic test for the identification of copy number changes in patients with mental retardation and/or congenital anomalies. The identification of a causative CNV is not only important to make a proper diagnosis but also to enable the accurate estimation of the recurrence risk to family members. The recurrence risk for *de novo* CNVs is considered very low, especially for *de novo* interstitial submicroscopic CNVs. At the macroscopic level insertional translocations occur only in approximately 1:80000 live births. However, despite of a substantial number of sporadic reports, the frequency of submicroscopic insertional translocations remains unknown.

To determine the frequency by which insertional translocations occur, we investigated the potential presence of an insertional translocation in a consecutive series of 326 *de novo* CNVs, including both deletions and duplications. For deletion, FISH with a probe within the CNV was performed on metaphase from both parents to determine whether one of the parents is carrier of an insertional translocation. For duplications, FISH was on metaphases of the index patient to determine whether the duplication was in tandem or on another chromosome. In the latter case, both parents were tested for the presence of an IT. In three (0.9%) parents a balanced, cryptic interchromosomal insertional translocation was detected.

This study shows that submicroscopic insertional translocations are much more frequent than microscopically visible ITs. This knowledge should be taken into account during counselling family members and might trigger follow up diagnostic tests in the parents.

P03.065 Two girls with mental retardation and behavioural abnormalities: Is the deletion of the *ATXN1* gene on 6p22.3 a major factor in causing the phenotype?

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6p22 interstitial deletions are relatively rare, with only eight patients described so far. The clinical phenotype in these patients includes developmental delay/mental retardation, defects of brain, heart and kidney, craniofacial malformations and dysmorphic features. The common deleted region, 2.2 Mb, harbouring 12 genes, has been defined as critical for this syndrome. *ATXN1*, located within this interval, has been suggested as a candidate gene for the mental retardation based on its expression in CNS and the cognitive impairments manifested in knock out mice. We report on two patients carrying partly overlapping *de novo* deletions within 6p22.3, which are smaller than the ones previously reported. Patient 1 displays mild mental retardation and behavioural problems; her 115 kb deletion (chr6:16215803-16332297, hg19) encompasses only *GMPR* and *ATXN1*. Patient 2 has severe mental retardation, autistic features and hyperactivity. Her deletion spans 2.3 Mb (chr6:14545576-16846846, hg19) and includes *JARID2*, *DTNBP1* and *MYLIP*, in addition to *GMPR* and *ATXN1*. Because the GMP reductases encoded by *GMPR* and *GMPR2* have redundant functions, haploinsufficiency of *GMPR* is not likely causing the cognitive impairments in our patients. We therefore suggest that *ATXN1* hemizygosity is sufficient to cause the mental retardation in patient 1. Furthermore, additional genes such as *MYLIP*, which is expressed in brain and has been shown to have a role in regulating neurite outgrowth, is expected to contribute to the significantly more severe phenotype of patient 2.

P03.066 Inverted duplication associated with an uncommon 1p36 interstitial deletion: description and mechanism**

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Inverted duplications with terminal deletion are rare chromosomal rearrangement. They have been described for a greater number of chromosomal ends since the development of molecular techniques. We report the first case of an inverted duplication associated with an interstitial deletion of chromosome 1 in a 23 years old woman with mental retardation, dysmorphic features and epilepsy. The cytogenetic analysis shows a normal female karyotype. The analysis with BAC array-CGH shows a chromosomal rearrangement consisting of a deletion and a duplication of the short arm of chromosome 1. The analysis with oligonucleotide array-CGH led us to the delineation of a 5 Mb duplication and 3.2 Mb deletion. A two color *in situ* hybridization allowed us to demonstrate for the first time an inverted duplication with an interstitial deletion. An Affymetrix SNP array discovered an uniparental disomy distal to the rearrangement. Two mechanisms were suggested to explain these chromosomal rearrangements:

- non allelic homologous recombination, the main mechanism in the inverted duplication with terminal deletion of the 8p. This abnormality is characterised by a single copy region in between the deletion and the duplication and by an inverted LCR or a micro paracentric inversion 8p in one of the parent.
- U-type exchange, characterised by the absence of a disomic region. Neither of these mechanisms can explain the formation of the inverted duplication with a interstitial deletion which leads us to expose a third possibility: a replication abnormality like FoSTeS (Fork stalling and template switching).

P03.067 A case with Turner-like phenotype carrying iso(Y)/monosomy X mosaicism

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Sexual disorders are associated with various numerical and structural aberrations of sex chromosomes. As for Y chromosome, isochromosomes are the most commonly reported abnormalities. Here we report a 14-year-old girl with short stature, Turner-like phenotype and amenorrhea. Her medical history was not remarkable. She was the second child of healthy non-consanguineous parents and had two healthy sisters. Her psychomotor development was normal while height and weight were below 3rd centile. Physical examination showed no axillary hairs and her breast development was TANNER Stage 1. Plasma levels of estradiol, FSH and LH indicated hypergonadotropic hypogonadism. Her bone age was 2 years below the chronological age. Abdominal MRI for uterus and ovaries showed that uterus was hypoplastic but no ovaries were visualized. Karyotyping with standard GTG banding revealed mos46,X,iso(Y)(qter-->p11.1-->qter)[20]/45,X[11]. C banding and subsequent FISH analysis with X and Y centromeric probes supported karyotyping. SRY analysis and laparoscopic exploration for redundant testicular tissue are planned.

P03.068 Chromosomal imbalances are an infrequent cause of non-syndromic sporadic congenital heart defects**

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Array Comparative Genomic Hybridization leads to an increased detection of causal chromosomal imbalances in individuals with syndromic congenital heart defects (CHD) (Thienpont *et al.*, 2007). This study explores the incidence of causal imbalances involved in non-syndromic sporadic CHD. High resolution SNP array was performed in 44 sporadic patients with an isolated and complex CHD (univentricular heart or atrioventricular septal defect (AVSD)). SNP array experiments were conducted, as described (Korn *et al.*, 2008). Genotypes were compared to a home-made reference model. Variants <10kb or with a >90% CNP overlap were not further investigated. CNVs which com-

prise chromosomal regions or genes known for causing CHD, were considered causal. The remainder were regarded as unclassified variants (UCV) with variable degree of causality based on gene-content and inheritance (Breckpot *et al.*, 2010).

101 rare variants were detected in 37 out of 44 patients. A *de novo* 22q11.21 duplication in one patient with AVSD was considered causal. We detected on average 2 UCVs per patient. Mean size of the detected UCVs was 83kb (median 31kb), comprising a mean of 52 markers per UCV (median 19).

Unlike previous studies (>10%, Greenway *et al.*, 2009), we demonstrate that chromosomal imbalances are an infrequent cause of sporadic, non-syndromic CHD. The 22q11.21 duplication was considered causal, since several independent studies showed an association with various developmental disorders including isolated CHD. Additionally, we detected a large number of UCVs in this patient cohort. Further studies are ongoing to assess the relevance of these unique variants in the pathogenesis of CHD.

P03.069 Phenotype-genotype correlation in a Tatar ethnic child with Kabuki-like features

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We report a 1-year-and 4 months-old girl, Tatar ethnic child, originating from South-Eastern region of Romania, with a phenotype reminiscent of Kabuki syndrome, mild developmental delay, long palpebral fissures, short stature. In 14 of 50 GTG-banded metaphases obtained from peripheral blood lymphocytes, a marker chromosome smaller than a G group chromosome was found. Fluorescence *in situ* hybridizations (FISH) showed the marker to be of X-chromosome origin. Also molecular analysis of the patient's genetic material confirmed the absence of the SRY gene. Proband's karyotype was described as mos45,X[36]/46,X,mar(X)[14]. Karotypes of the parents were normal. Other molecular studies will be performed. We present our case comparatively with another published Kabuki-like cases with sex chromosome abnormalities.

P03.070 Simultaneous copy number and loss of heterozygosity detection using Agilent's CGH+SNP platform

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In the past few years, genome-wide studies have broadened the need for simultaneous detection of genomic variations and allelic imbalances. Single nucleotide polymorphism (SNP) measurements can complement DNA copy number (CN) measurements to enable detection of allelic gains and copy-neutral changes such as loss of heterozygosity (LOH) and uniparental disomy (UPD).

The current SurePrint G3 Human CGH platform was extended to include a set of SNP probes on the same microarray, allowing simultaneous DNA Copy Number (CN) and Loss of Heterozygosity (LOH) detection. Using the standard enzymatic labeling protocol, we evaluated the CN and LOH state in a set of cytogenetic samples with previously characterized disorders. The genomic DNA was restriction digested with AluI and RsaI, allowing detection of SNPs located in the enzyme recognition sites. Following hybridization of the labeled gDNA to CGH+SNP microarrays, the data were analyzed using Agilent's Genomic Workbench software.

Several LOH regions, as small as 5.8 Mb, were detected in different samples. A trisomy of chromosome 21 and a duplicated region on chromosome X were identified in two samples. The assay correctly assigned known partial and complete UPDs. In one case, extended segments of homozygosity were found throughout the genome as a consequence of parental consanguinity. In two additional cases, UPD was associated with individuals known to have Angelman and Prader-Willi syndromes, respectively. Our CGH+SNP platform confidently and accurately identified and mapped allelic gains, LOH and UPD aberrations, previously reported as associated with the samples studied.

P03.071 Molecular and clinical characterization of 6q16 deletions encompassing *MCHR2*, *SIM1* and *GRIK2* genes in 3 patients with mental retardation +/- high weight and small extremities

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Deletions of the chromosomal segment 6q16 have been reported in association with a Prader-Willi like phenotype. Array comparative genomic hybridization enables to accurately define the region of interest and five cases have recently been reported by Bonaglia *et al* (2008), allowing them to narrow the minimal critical region to a 4,1-Mb segment.

Here we describe three more cases of overlapping 6q16 deletions. By comparing these deletions with the previously reported 4,1-Mb region, we define a new minimal deleted fragment of 2,2 Mb (100,489,030 ->102,689,000)(hg18-build36) involving four genes: *MCHR2* encodes a receptor for melanin-concentrating hormone which is involved in the control of feeding behaviour and energy metabolism; *SIM1* (candidate gene for obesity), a transcription factor that plays an important role in the development of the paraventricular neurones; *ACSS3*, an helicase; and *GRIK2*, a glutamate receptor which could be involved in autistic-like behaviour.

The three patients reported here present a moderate developmental delay with language retardation.

Two of them have also a high weight and even obesity in accordance with haploinsufficiency of the *SIM1* gene, as well as a rounded face and small extremities. On the contrary, the third patient has an average weight and normal extremities, but he presents a complex chromosomal rearrangement with two deletions of the long arm of chromosome 6, in 6q16.1 and 6q16.2q16.3. Moreover, the deleted chromosome 6 is involved in a balanced translocation (6 ;13)(q16.1q21).

The phenotypic difference could be explained by expression alteration of another gene, alteration which would offset haploinsufficiency of *SIM1*.

P03.072 Mental retardation in a patient with 14q32.33 and 19p13.3 microdeletions characterized using array-based CGH

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A 17-year-old boy was referred to the Centre for Medical Genetics because of abnormal gait and distinctive phenotype. It consists of moderate mental retardation and dysmorphic features including narrow palate, sparse eyebrows, scoliosis and broad feet. The conventional cytogenetic analysis revealed a normal 46,XY karyotype. Array-based comparative genomic hybridisation (aCGH) analysis demonstrated the presence of a 0,4 Mb deletion in 14q32.33 and 0,4 Mb deletion in 19p13.3. The deleted regions encompass 26 genes, among which at least 3 candidate genes for genotype-phenotype correlation could be delineated. The products of TMEM121, GPR54 and KISS1R genes are expressed in the developing brain and could possibly be related to mental retardation. Additional patients with well-characterized deletions within these regions will be needed to determine the role of individual genes for the clinical manifestations.

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P03.073 Report of two dysmorphic and mentally retarded cousins with identical inherited derivative chromosome 1 with different degrees of clinical severity

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Two paternal cousins were referred for genetic counseling due to abnormal clinical features and mental retardation. The first was a 3 year old female from a consanguineous marriage and the other was a 4 year old male and result of a non-consanguineous marriage. Both mothers had a history of miscarriages. Cytogenetic studies revealed an abnormal karyotype, described as der(1)t(1;9)(q42;q32). In the first patient, the abnormal chromosome was inherited from her father and the other from his mother. Both these parents had the same balanced reciprocal translocation between chromosomes 1 and 9, described as t(1;9)(q42;q32).

The two patients had similar clinical features including mental retardation, microcephaly, speech and developmental delay, arachnodactyly, asymmetric face, asymmetric skull, short filtrum and high arched palate and female patient had a history of seizure up to 1.5 years. However, the clinical features in the male patient were more severe than the female patient. To our knowledge, this is the first reported case.

P03.074

Structural rearrangements of chromosome 8p23.1-p23.3 related to mental retardation

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Mental retardation (MR) which occurs in about 1-3 % of population is etiologically very heterogeneous and unexplained in more than a half of all cases. Rearrangements in 8p are frequent and associated with MR. A unique feature of the chromosome 8 is a vast region of 15 Mb on distal 8p that appears to have a strikingly high mutation rate. Three major factors have been associated with high mutation rates: proximity to telomeres, high recombination rate and high A+T content.

We identified one duplication and five deletions in 8p23.1-p23.3 region using Infinium-2 whole-genome genotyping HumanCytoSNP-12 Bead-Chips (Illumina) and arrayCGH (Agilent 105K) in 38 patients with MR. Rearrangements size varies from 500 Kb to 6.7 Mb. 6.7 Mb 8p23.1-23.3 duplication was identified in 11 years old boy with MR, developmental delay, macrocephaly, speech disorders. Region of 8p23.1-23.3 contains genes which may be related to neurological abnormalities: *CLN8*, *DLGAP2*, *MCPH1*. Gene *CLN8* encodes a transmembrane protein which is postulated to function in lipid synthesis, transport, or sensing. Mutations in *CLN8* gene have altered levels of sphingolipids and phospholipids in the brain and are associated with progressive epilepsy with MR which is a subtype of neuronal ceroid lipofuscinoses. *DLGAP2* gene encodes a membrane-associated guanylate kinase that is localized at postsynaptically density in neuronal cells and may play a role in the molecular organization of synapses and in neuronal cell signaling. Additional copies of *MCPH1* gene have been associated with macrocephaly.

P03.075 SNPs-array analysis in 100 patients with mental retardation

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The underlying genetic defect remains difficult to diagnose in the majority of patients suffering from mental retardation. Recent developments in genomic microarray technology now allow for the genome-wide detection of submicroscopic chromosomal alterations. The SNPs array currently represents the technique with the highest resolution power, since they allow to identify chromosomal abnormality as for example variations in the number of copies (CNV) as duplications or deletions and uniparental disomy (UPD). We have collected 100 samples from patients with normal karyotypes and some samples from their unaffected family members. The patients presented moderate or severe MR, associated with at least one of the following clinical features: one major malformation and/or dysmorphism and/or multiple minor anomalies. We have applied Infinium-2 genotyping assay with Human370CNV-Duo or Quad BeadChips (Illumina Inc.) to detect CNVs and copy-neutral LOHs.

The data was analyzed with GenomeStudio 2009. We developed an ad hoc database to maintain all relevant information.

The Illumina SNP platform detected CNVs most of which have already been reported in the Database of Genomic Variants or recurrently present in our samples. We shall discuss unique CNVs and their associated phenotype.

Patients with CNVs, who weren't identified in the database or recurrent in our cases, have been submitted to further investigation (FISH or SNPs array on their parents). The results show that SNPs array can be used to reliably detect and characterize subtelomeric and interstitial CNVs which are the cause of the pathological phenotype.

P03.076 Novel cryptic genomic aberration associated with mental retardation

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Expanding usage of whole-genome genotyping arrays in molecular cytogenetics has given a new quality to molecular karyotyping of patients with developmental abnormalities e.g. mental retardation (MR). In addition to high resolution of SNP-CGH arrays, the possibility to combine copy-number variation (CNV) data with genotyping information provides an advantage to use array data for more widespread analysis. It allows to detect uniparental disomy and copy-number neutral loss of heterozygosity tracks on genome-wide level as well as to characterize allelic composition and inheritance pattern of found aberrations or to use the same information in whole-genome association studies for relating smaller rare CNVs with phenotypic features in patients.

Here we report a cohort of Estonian patients with idiopathic mental retardation screened for genomic structural aberrations - CNVs, inconsistencies of mendelian inheritance as indicative for potential uniparental disomy (UDP) and rare extended runs of homozygosity (ROH) - by Infinium whole-genome genotyping assay with HumanCNV370 arrays (Illumina Inc.). We found structural aberrations with potential clinical significance in 23% of investigated families, including seven cases with recurrent microdeletion or -duplication syndromes and eight families with imbalances overlapping with previous similar reports. Four novel aberrations with probable clinical significance were identified in chromosome regions 2p25.1-p24.3, 3p12.1-p11.2, 7p21.1-p21.1 and Xq28. Additional four CNVs less than 200kb in size and two extended ROHs with potential, yet uncertain role in the pathogenesis of MR were detected.

P03.077 Xp22.3 interstitial deletion: a clinically recognisable genetic condition identified by array comparative genomic hybridisation

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X linked ichthyosis is a genetic disorder affecting the skin and is caused by a deficit in the steroid sulfatase enzyme (STS). This syndrome is often associated with a recurrent microdeletion at Xp22.31.

Most of the STS deleted patients have X linked ichthyosis as the only clinical feature and it is believed that patients with more complex disorder, including mental retardation are the result of contiguous gene deletion .

This lead to the hypothesis that mental retardation gene should be present within this microdeletion. It was previously proposed the VCX-A, a member of the VCX (variably charged X-chromosome mRNA) gene family, as the candidate gene for X-linked non-specific mental retardation in patients with X linked ichthyosis.

Using array comparative genomic hybridization (CGH array) on a 44K resolution, we identified an interstitial deletion of Xp22.3 of 1, 32 Mb (oligonucleotide position: from A-14-P139110 to A-14-P125042), including the STS, VCX (A, B, 3A and 3B) and HDHD1A genes.

The phenotype observed in our male patient had X linked ichthyosis, moderate mental retardation, facial dysmorphic features and epilepsy. Many previous results, suggest that VCX-A gene is sufficient to maintain normal mental development. Thus, our findings support a causal role for

VCX-A dysfunction in X linked ichthyosis patients who manifest mental retardation.

P03.079 Use of the Affymetrix Cytogenetics Whole-Genome 2.7M Array in routine molecular karyotyping: first experiences and results

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Microarray analysis has become an increasingly powerful tool for the detection of submicroscopic chromosomal imbalances. Previously, we used the Affymetrix 500K and the Genome-Wide 6.0 SNP arrays for research purposes and now established the Cytogenetics Whole-Genome 2,7M Array for routine molecular karyotyping. This array contains 2,3 million copy number and 400.000 SNP markers allowing the detection of copy number alterations, the loss of heterozygosity and uniparental disomy in a single run.

So far, we analysed 24 patients with this array: in 5 patients chromosomal aberrations previously detected with the 6.0 array, aCGH or conventional karyotyping were confirmed. Another 19 patients showed no chromosomal aberrations.

The cytogenetics array offers several advantages in comparison to other array techniques: in contrast to aCGH a control DNA for each sample is not necessary. Instead, a reference of 450 pre-analysed HapMap samples can be used for computation. Compared to the 6.0 array the workflow is much faster with less hands-on- and total time. The Chromosome Analysis Suite (ChAS) software provided by Affymetrix is easy to use, results are clearly arranged and aberrations are directly linked to databases e.g. UCSC genome browser and Ensembl. Nevertheless there are some disadvantages, too. The software sometimes shows computation biases: results may be false positive due to the use of different annotation files especially regarding the sex chromosomes and due to poor marker-coverage in some regions. Nevertheless, the cytogenetics array is a powerful, fast and reliable tool and suitable for routine molecular karyotyping.

P03.080 A boy with mental retardation, obesity, dysmorphism and hypertrichosis caused by a microdeletion of 19p13.12.

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We present a moderately mentally retarded boy with obesity, short stature, hypertrichosis and facial dysmorphism.

Using the Illumina Bead Array platform a *de novo* deletion of chromosome 19p13.12 was identified in DNA from our patient. Aberrations of the gene-rich chromosome 19 have rarely been reported. The deletion in our patient is approximately 1.2Mb in size, with breakpoints at 14.2Mb and 15.4Mb, and encompasses 30 genes (10 OMIM-listed). It includes NOTCH3 (MIM600276), causative of the vascular neurodegenerative disorder CADASIL. In CADASIL, pathogenic mutations alter the number of cysteine residues in the extracellular domain of NOTCH3, which accumulates in small arteries of affected individuals. It has recently been suggested that CADASIL mutations act through gain of function mechanisms. As mutations in NOTCH3 play a role in neurodegeneration, it is tempting to speculate that haploinsufficiency of this gene might contribute to the cognitive impairment in our patient. Another gene potentially related to the phenotype in the deleted region is the CASP14 gene (MIM 605848), playing a central role in apoptosis. It is highly expressed in the inner root sheath of the hair follicle and haploinsufficiency of this gene may well contribute to the hypertrichosis in our patient.

P03.081 Patient with minor dysmorphism and microdeletion/microduplication confirmed by aCGH due to the presence of a double translocation

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ArrayCGH is the technique, day by day adds new syndromes of microduplication or microdeletion in situations of mental retardation and/or congenital anomalies. We report a new syndrome with a double chromosomal aberration: microdeletion and microduplication in a

case investigated for mental retardation, growth retardation and minor dysmorphic features (microcephaly, downwards slanting palpebral fissures, retrognathia, 2nd and 5th finger clinodactyly of both hands and umbilical hernia). A double chromosomal translocation, one involving chromosomes 1 and 13, and the other involving chromosomes 5 and 11 was revealed by the conventional cytogenetic analysis performed from peripheral blood lymphocytes with GTG banding. For establishing the origin of the translocation, chromosomal analysis was performed for both parents, but their karyotypes were normal and we concluded that is a de novo translocation. Due to the presence of phenotypic manifestations and the presence of mental retardation we suspected a possible genomic imbalance. ArrayCGH using 105A Oligo Microarray kit was performed for our patient and revealed a 2,3Mb deletion on 13q31.3 and 1Mb duplication on 2q14.3. Application of genome wide array for detection of chromosomal aberration is mandatory in clinical practice because it allows the correct characterisation of genomic imbalance. Other literature reports stated that when performing aCGH for patients with translocations, genomic imbalance of other chromosomes than the ones involved in translocation might be revealed. This applies to our case, as a microduplication was found on chromosome 2 that is not involved in translocation.

P03.082 Association of aberrant promoter methylation of checkpoint genes in miscarriages with chromosomal mosaicism that fail to grow *in vitro*

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Chromosomal abnormalities play a significant role in embryo development failure. About 50-70% of them are found in mosaic state with diploid cells. The molecular bases of chromosomal mosaicism remain unknown. In cancer studies hypermethylation of cell cycle control genes was shown, what potentially can cause chromosome missegregation. We aimed to analyze karyotype of 18 embryos with cell culture failure and 44 aneuploid miscarriages after conventional cytogenetic analysis using FISH with centromere-specific DNA probes and to study epigenetic status of M (*CHFR* and *MAD2*) and G1/S (*RB1* and *p14*) checkpoint genes with methyl-specific PCR. Two extraembryonic tissues (mesoderm and cytotrophoblast) were studied when available. Mosaicism was more frequently observed among miscarriages with cell culture failure ($p<0.01$). Cytotrophoblast-specific compartmentalization of aneuploid clones prevailed in this group, what testify to the higher rates of mitotic mutations in cytotrophoblast and trisomy rescue in mesoderm. In successfully cultured embryos abnormal cells prevailed in mesoderm. The frequency of *p14* and *RB1* hypermethylation was significantly higher in miscarriages with cell culture failure - 83% vs. 7% in successfully cultured ($p<0.01$) and it was mainly observed in mesoderm or in both tissues. There was no *CHFR* and *MAD2* hypermethylation in both groups. Our findings suggest that there is an association between hypermethylation of G1/S checkpoint genes and mitotic errors. Also prevalence of epimutations in cell culture failures may indicate their ability to reduce developmental potential of embryos. This study was supported by grant of Federal Program #P806.

P03.083 MLPA analysis in children with mental retardation

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Chromosomal aberrations are the most common known cause of mental retardation. However, the typical rearrangement is below a resolution limit of standard cytogenetic banding. MLPA is a modern molecular genetics method of choice that can detect such small deletion/duplication in a number of selected regions at a time.

Our sample was made of 41 unrelated probands in charge of the Department of Medical Genetics (GTH and 1stFM, Charles University in Prague). Selection criterions were the presence of mental retardation corresponding to cryptic rearrangements but without a positive cytogenetic finding on a standard cytogenetic karyotyping. We have analysed all probands using MLPA kits P245 for detection of 21 most common deletion/duplication syndromes, P036 for the subtelomeres analysis, and P297 designed to examine new chromosomal regions recently associated with mental retardation.

Overall, duplication and five various deletions were obtained (12%). All positives were detected by the P245 MLPA kit. In one case the deletion covered subtelomeric region and was also traced using the MLPA kit P036. No additional positives were after the P297 MLPA kit analysis. We found the MLPA method as a fast, cheap, and effective method to analyse patients with mental retardation within the laboratory routine. *This work was supported by Grant No. IGAMZCR NS/10327-3/2009.*

P03.084 Array CGH; an excellent tool in the search for low-level mosaics.

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Department of Genetics; University Medical Centre, Groningen, Netherlands. Somatic chromosomal mosaicism is a well established cause for mental retardation and multiple congenital abnormalities (MR/MCA). However, low level mosaicism for clinically relevant chromosomal abnormalities can be missed if masked by a high percentage of normal cells or due to selective growth of normal cells in culture. To overcome this problem, either the number of karyotyped cells is extended and/or interphase FISH in uncultured tissues is performed. With the introduction of array CGH techniques analysis of uncultured cells is easily feasible and up to 10% mosaicism is detectable (Cross et al, 2007).

In our cohort of 1730 MR/MCA patients analyzed by array CGH (either 105K or 180K custom designed Agilent oligo arrays) and by karyotyping (10 metaphases), array CGH analysis detected 12 mosaics. Only 5 of these were detected by karyotyping. Mosaics included four trisomies (chromosome 13, 14, 18, 20), two monosomies (chromosome X, 21), four extra markerchromosomes (chromosome 8, 12, 14, 18) and two submicroscopic structural abnormalities (duplication 1q32.1, deletion 1p36.32p36.31). For each case confirmation by FISH analysis was performed on PHA stimulated blood cells and on a second tissue if available. The percentage of mosaicism ranged from 1 to 80%. In our study, karyotyping failed to detect seven out of twelve (58%) chromosomal mosaic cases and these included low level as well as high level mosaicism. We conclude that array-CGH is an excellent tool for detection of any type of mosaicism and therefore the preferred choice of technique.

Cross et al. 2007. *Prenat diagn* 27,1197-1204

P03.085 Use of buccal smears to investigate tissue specific mosaicism

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The suspicion of possible chromosomal mosaicism, the presence of two or more chromosomally different cell lines within an individual, can lead to diagnostic as well as genetic counselling dilemmas. It is well documented that levels of mosaicism can vary greatly within different tissue types from the same patient and hence it is clinically advantageous to study tissues of differing embryonic origin. Obtaining smears from buccal swabs is a rapid and relatively non invasive technique for obtaining skin fibroblasts, which is in contrast to the invasive and time consuming procedures such as taking skin biopsies. Buccal smears facilitate the rapid identification of identified or suspected chromosomal abnormalities using interphase FISH, although their disadvantage is that G-band analysis is not possible. We report the FISH analysis on buccal smears from 15 patients to investigate possible chromosomal mosaicism following G-band analysis on at least one other tissue type, most commonly peripheral blood. The presence of mosaicism was demonstrated in four of the 15 patients. Our findings included confirming a diagnosis of suspected diploid/triploid mosaicism, with the detection of a triploid cell line present in approximately 40% of fibroblast cells. Another patient was found to have a mosaic cell line within the skin tissue containing a structurally rearranged chromosome 18. Notably, in both these instances the peripheral blood karyotype was normal. These results demonstrate that buccal smears provide a valuable and easy to obtain source of material to investigate for possible tissue specific mosaicism.

P03.086 High-resolution 44K array and 1 Mb array detect similar rates of chromosomal imbalances in patients with müllerian defects (MD)

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The defective development of the Müllerian ducts results in abnormalities of the female genital tract ranging from upper vaginal atresia to total absence of Fallopian tubes, uterus and upper vagina, while ovaries, breast development and patterns of body hair are normal. Patients often exhibit additional clinical features such as renal, vertebral and cardiac defects. In most cases its etiology remains poorly understood. The vast majority of patients have a normal female karyotype and few individuals have been found to carry chromosomal abnormalities. Recently our group investigated by 1Mb BAC array CGH patients with MD and chromosomal imbalances in 33% of them affecting the 1q21.1, 17q12, 22q11.21 and Xq21.31 chromosome regions. In order to verify whether smaller genomic alterations contributed to the MD etiology, we investigated 15 patients previously screened by BAC array and presenting normal results. For this study, we used a CGH microarray platform from Agilent Technologies containing 44.000 oligonucleotides, i.e., over 10 times higher resolution than the array previously used. Only copy number variations of DNA segments which are also observed in control populations (i.e. without a major pathogenic effect) have been detected among our patients. A possible explanation for these negative results is that the patients of the first study were more syndromic than the remaining patients and presents more additional clinical signs such as renal, craniofacial and member alterations and learning disabilities. Although the size of the sample is still limited, our results indicate that array-CGH has little diagnostic value for patients with isolated müllerian defects.

P03.087 X-chromosome disorders: identification of underlying mechanisms

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Non-allelic homologous recombination (NAHR), nonhomologous end joining (NHEJ), and recently Fork Stalling and Template Switching (FoSTeS) have been implicated as the main mechanisms for the creation of genomic disorders. In order to investigate the mechanisms responsible for the creation of X-chromosome disorders we analyzed 70 cases bearing cytogenetically visible X-chromosome abnormalities using whole genome tiling path BAC arrays and custom designed targeted ultra-high resolution oligo-arrays. Using whole genome tiling path BAC CGH, we were able to accurately map the breakpoints of 35 cases bearing isochromosomes of the long arm of chromosome X at a resolution of 150kb. Fifteen of these had breakpoints at the centromere and were considered monocentric. The remaining 20 were isodicentric and had breakpoints in proximal Xp in ChrX:51500000-58500000. This region of chromosome X is rich in segmental duplications and contains some of the largest and most homologous inverted repeats in the human genome. Based on the BAC-array CGH findings, we designed custom oligo-arrays which cover this region in ultra-high resolution (44K and 385K oligos in 7Mb region of interest) and feature enhanced coverage of segmental duplications. Screening the isodicentric cases with these ultra-high resolution arrays enabled us to identify previously undiscovered breakpoint complexity in 45% of the isodicentrics and demonstrate that they are formed by NAHR, facilitated by specific highly homologous inverted repeats. Twenty two percent of the isodicentrics were mapped within repetitive sequences and 33% have simple breakpoints that do not coincide with segmental duplications and are probably mediated by a nonhomologous recombination mechanism.

P03.088 Common polymorphism near the IL-6 gene is associated with total and abdominal adiposity in Caucasian men: 5-year follow-up study in Swedish GOOD cohort

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Background: Regulation of fat mass is associated with immune functions. Studies of knockout mice show that endogenous interleukin (IL)-6 can suppress mature-onset obesity. Our hypothesis is that variants of the IL-6 gene can affect obesity in humans. We recently published results revealing association between the SNP rs10242594, mapping slightly downstream of the IL-6 gene, in GOOD cohort, consisting of 1049 men aged 18-20 years ($p=.02$). This finding was confirmed in elderly men (nearly 8000 in total; Andersson N et al 2010, *Int J Obes*). Later on, this association was confirmed and strengthened in the GOOD GWAS, after exclusion of individuals with non-Caucasian descent ($p=.002$, unpublished data). In this study we aimed to examine the influence of rs10242595 on obesity in a 5-year follow-up study of the GOOD subjects.

Study Design: 724 participants of the GOOD-cohort, now 23-25 years of age, were available for follow-up measurements of body fat by DXA. Association of rs10242595 was analyzed by linear regression and adjusted for age, length, tissue lean mass and current physical activity.

Results: rs10242595 is significantly associated with fat percent ($p=.016$), total body fat mass ($p=.012$) and abdominal fat ($p=.01$) in young Caucasian men investigated 5 years after the first measurement. **CONCLUSION:** These findings indicate that rs10242595*A is associated with decreased abdominal fat as well as total fat in young men. We hypothesize that another, still unknown, polymorphism mapping to the coding region of the IL-6 and in strong LD with rs10242595 is one of the causing genetic factors of obesity.

P03.089 Pericentric inversion of chromosome 9 in medical genetics and clinical counselling

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Pericentric inversion of chromosome 9 [inv(9)] is mostly considered to be clinically insignificant variant of the human karyotype. However, several studies have shown possible connection of inv(9) with certain groups of clinical diagnoses: infertility, repeated abortions or schizophrenia.

In our retrospective epidemiological study, we have analysed the incidence of inv(9) and the spectrum of clinical indications for karyotyping in three medical genetics departments in Prague. In cytogenetic archives of these departments (at General Teaching Hospital, Thomayer University Hospital and Pronatal Sanatory) we identified 351 cases with inv(9) (1.60 %) among 21 944 cases in total (reference period: 1981 - 2008). The sex ratio (female to male) in inv(9) patient group was 1.29. A special control group of 515 patients with normal karyotype was created from the cytogenetic archive in the General Teaching Hospital, using the systematic sampling method.

The most frequent clinical diagnoses among the patients with inv(9) were: infertility and/or habitual abortion - 124 cases (37.13 %), congenital anomalies - 41 (12.28 %) and psychomotoric and/or mental retardation - 24 (7.19 %). Comparing these incidences with those in the control group we determined the relative risk of infertility and/or habitual abortion for inv(9) carriers as 1.21 (95% CI = 0.90 - 1.58); $p = 0.1726$ (Fisher's exact test). As this result was not statistically significant we haven't proved any strong association of inv(9) with specific clinical diagnosis and the clinical significance of inv(9) still remains uncertain. The molecular-genetic examination of inv(9) itself should be our next step.

P03.090 Atypical variant rearrangement bearing a BCR/ABL fusion gene on the chromosome 7 in a case of chronic myeloid leukemia with a translocation t(7;9;22)(q11; q34; q11)

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Chronic myeloid leukemia (CML) is characterized by the presence of Philadelphia chromosome, usually resulted from a t(9;22)(q34;q11). Approximately, 5-10% of CML patients show variant translocations involving other chromosomes in addition to chromosomes 9 and 22. In some variant translocations, additional material is transferred on the derivative chromosome 22 resulting in a masked Philadelphia chromosome. In this study, we report an apparently masked Philadelphia CML patient showing a t(7;9;22)(q11; q34; q11) by conventional cytogenetic and a b2a2 transcript by multiplexed polymerase chain reaction. A molecular cytogenetic characterization was performed by fluorescence in situ hybridization (FISH) and disclosed the presence of the BCR/ABL fusion gene on the derivative chromosome 7. FISH analyses with whole chromosome painting specific for chromosomes 7, 9 and 22 allowed us to precisely characterize the variant chromosomal rearrangement that was not perceived by conventional analysis and to discuss the mechanism formation of this chromosomal rearrangement. Thus, resulted from a sequence of rearrangements (two steps): the first rearrangement is a classical t(9;22)(q34;q11) which places the BCR/ABL fusion gene on the derivative chromosome 22, this reciprocal translocation is followed by a second rearrangement between derivative chromosome 22 and chromosome 7, giving rise to a BCR/ABL fusion gene on the derivative chromosome 7.

In summary, our data provide a precise molecular cytogenetic characterization of a Philadelphia negative patient, bearing variant t(7;9;22), we also highlight the crucial role of the FISH analyses in disclosing masked Philadelphia chromosomes with is beyond the power resolution of conventional cytogenetic.

P03.091 Molecular and cytogenetic studies of the Prader-Willi syndrome in Romania

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This study is part of the research introduced in Romania for PWS patients. Multidisciplinary physical examination allows the correct establishing of the clinical score. The strategy we propose for the confirmation of the clinical PWS diagnosis includes initially a methylation analysis (MSPCR). This test is used as a diagnostic instrument for PWS because methylation pattern is parental specific in this region and detects patients with deletions, UPD and imprinting defects, that represent 99% of PWS cases. FISH technique, using specific probes for the 15q11-q13 region, is considered as the most efficient cytogenetic diagnostic method for PWS, identifying the deletion in approximately 80% of patients. In our preliminary study, only 5 of the 11 patients (45,5%) had a positive FISH test. Because FISH doesn't detect UPD and mutations of the imprinting center, the rest of the patients need a DNA investigation using microsatellite markers inside and outside PWS region.

In conclusion, the results obtained in the study group, even if its' size doesn't allow important statistic conclusions, differ from those reported in the specialized literature, both in the proportion of PWS cases confirmed by methylation analysis (70% compared to 99%), and that of cases confirmed by FISH analysis (45,5% compared to 70%). The explanations could be related to a particular molecular profile of PWS patients in Romania. Such studies do not exist for the moment in our country and the confirmation will be possible by investigating a bigger number of patients.

P03.092 The importance of invasive prenatal diagnostics results in genetic screening programme

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The purpose of the study was appraised an effectiveness of prenatal genetic screening by invasive diagnostics procedures.

1747 samples of fetus material (chorion's thief or placenta's cells and umbilical blood's lymphocytes) were materials for invasive prenatal diagnostics and cytogenetic analysis by period from 2008 to 2009 years in Almaty. Biopsies of chorion's thief were in 947 (54,2%) cases, placental centesis were in 589 (34%) cases and cordocentesis were in 145 (8,2%) cases, interphase/nuclear in situ hybridizationis in 66(3,8%). Samples were studied by G-karyotyping cytogenetic method.

Normal karyotypes of fetus were diagnosed in 1602 cases (91,7). Chromosomal pathologies were diagnosed in 79 cases by pregnant women of high-risk group (5%), that according with literature's dates. The more common indications for invasive prenatal diagnostics were pregnant women's age with US-markers (NT, nasal bone) and serum markers (PAPP-A, beta-CGH, alpha-fetoprotein).

Invasive prenatal diagnostics in 624 cases were made by isolate indication (36%). The highest rate of chromosomal pathology diagnostics by isolate indication was by ultrasonic 207(12%) and serum 502(29%) markers detections. The lowest rate was by only age indication 385(22%).

But combination of two and more indications was more important (170 cases, 10%): age factor + serum markers changes (92 cases) the rate of fetus chromosomal aberrations was 5,2%, age factor + ultrasonic markers - 2,3% (40 cases). The maximal rate of fetus chromosomal changes was by combination of serum and ultrasonic markers (38 patients - 2,1%).

The more common indications for invasive prenatal diagnostics were pregnant women's age with US-markers and serum markers.

P03.093 An Xp22.33 duplication flanking the pseudoautosomal region translocated on Yp11.2**

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The human pseudoautosomal regions (PARs) represent short regions of homology on the terminal parts of X and Y chromosomes that behave like autosomes and pair and recombine during meiosis. Upon array analysis of the DNA of a 13 year-old-boy with behaviour problems (Smith-Magenis like) and Ebstein anomaly a 129 kb Xp22.33 duplication was detected. Surprisingly, the duplication is inherited from his healthy father. FISH with a BAC clone from that region showed a signal on the Y chromosome. We hypothesize the duplication might represent an extension of PAR1. One possibility is that this is a de novo extension of the PAR by an insertional translocation. Considering that the duplication on X is reported in the normal population, it may also be an evolutionary ancient PAR. Further analysis is ongoing to distinguish between these possibilities.

P03.094 Chromosome translocation 46,XX,t(2; 5)(q13; q12.3-13) in a girl with psychomotor retardation

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We study a girl with severe psychomotor retardation product of normal, young, healthy and non consanguineous parents. She was born below the 3rd percentile. On physical examination she weighted and heighed below the 3rd percentile, she presented plagiocephaly, left occipital flattening, narrow forehead, low nasal bridge, flat facial profile, synophrys, narrow palpebral fissures, short nose, long philtrum, short neck, prominent anterior chest, aberrant right palmar crease and clinodactyly of 5th finger of both hands. Karyotype with G bands was 46,XX,t(2;5)(q13;q13). To our knowledge this is the first case in the literature of translocation between chromosomes 2 and 5 (q13, q12.3-q13). The patient has severe psychomotor retardation and dysmorphic changes probably attributed to one or more genes located on the site of rupture. More studies are needed to define more precisely the rupture sites in this case.

P03.095 Molecular characterization of r(18) chromosomes in four patients

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Ring chromosomes are usually associated with loss of chromosomal material at one or both arms of the chromosome and mitotic ring instability. Sometimes the rings are more complex with additional material gain. Ring chromosome 18 is a rare finding with about 70 cases reported so far. About 75% of these cases are female.

The clinical phenotype mainly depends on the size of the deletion. We report here on four cases, three females and one male, with a ring chromosome 18 first revealed by conventional karyotyping. Microsatellite PCR analyses indicated maternal and paternal origin in each two of them. High resolution Human Omni1-Quad (Illumina[®]) arrays were used to determine the breakpoints and the exact sizes of the deletions, which were between 1.6 and 15 Mb on 18p and 3.8 and 24 Mb on 18q, and between 16.6 and 38 Mb in total, respectively. One case was found to be complex with an additional 1.5 Mb duplication on 18p (p11.23->p11.31). Confirmation experiments as well as investigations to determine the parental origin separately for the deleted and duplicated segments and the mechanism of formation in the latter case are in progress.

Our cases confirm the previously observed high phenotypic variability for r(18), depending on the size of the aberrations. According to the literature, the majority of cases shows the clinical picture of the 18q-syndrome, i.e. del(18)(q21->qter). This holds also true for the case with the duplication, corroborating previous observations according to which duplications often remain without major clinical consequences.

P03.096 Genetic investigations on 8 patients affected by a characteristic seizure disorder associated with ring 20 chromosome mosaicism

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Mosaic Chromosome 20 ring [r(20)] is a chromosomal disorder associated with a rare syndrome characterized by a typical seizure phenotype, a particular electroclinical pattern, cognitive impairment, behavioural problems and absence of a consistent pattern of dysmorphology. The pathogenic mechanism underlying seizures disorders in r(20) syndrome is still unknown.

We performed a detailed clinical, cytogenetic, FISH, array-CGH and molecular study on 8 patients with ring 20 chromosome mosaicism, including two members of the same family.

Our results indicate that higher percentages of r(20) cells are related with precocious age at seizure onset and with resistance to antiepileptic drug treatment. Behavioural problems also seems to be associated with higher percentages of r(20) cells. FISH and array-CGH experiments failed to detect any cryptic deletion on chromosome 20. No evidence of chromosome 20 uniparental disomy was found. Analysis of FISH signals given by variant in size alphoid tandem repeats probes on the normal chromosome 20 and the r(20) in the mosaic carriers suggests that the r(20) is the same chromosome not circularized in the "normal" cell line.

According to this hypothesis an epigenetic mechanism perturbing the expression of genes close to the telomeric regions, rather than haploinsufficiency of genes located at the distal 20p and/or 20q regions, may underlie the manifestation of r(20) syndrome.

P03.097 Asynchronous replication of human subtelomeric regions

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Replication of human genome is linked to different basic cellular processes. Studies conducted so far have shown that timing of DNA rep-

lication correlates with genomic instability, transcriptional activity, gene density and chromatin structure. Synchronous replication of alleles was demonstrated for genes with a biallelic expression while asynchronous replication for those with monoallelic mode of expression due to imprinting, X-inactivation or allelic exclusion. Little is known about replication timing(RT) of subtelomeric regions(SR), and its correlation with chromatin structure and function remains poorly understood. SRs of human chromosomes are highly unstable, rich in genes and frequently involved in aberrations and thus are of particular clinical interest.

In this study the replication pattern of SR of chromosomes 2,5,7,8,9,16,19 and 20 was investigated using FISH method in lymphocytes of 8 healthy donors. At least 200 cells/probe/person were analyzed. The replication status of a locus was classified as unreplicated(s) and replicated(d).

The analysis revealed marked heterogeneity of investigated SR. The data suggested a continuum of replication throughout S phase, with SR9p (dd:20.9±0.96%) being the first, and SR2p (dd:3.7±0.51%) the last to replicate (p<0.01). The results showed asynchronous replication at some SR. The frequency of sd ranged from 18.6% to 33.2% and the mean for SR19q2q16q20q5p (sd:31.08±0.52%) was significantly higher (p<0.01) compared to SR2p7q5q (sd:19.9±0.58%).

This investigation revealed that different SR replicates at different times during S phase and that certain homologous loci replicate asynchronously. Although the significance of RT is unclear yet, it might contribute to the non-random involvement of specific SR in chromosomal rearrangements.

P03.098 Detection of molecular cytogenetic damage of individuals affected by retinoblastoma in south India

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Retinoblastoma is the most common primary cancer of the eye among children and has both hereditary and sporadic form. The aim of the present study was to analyze the chromosomal analysis as a marker for diagnosing the disease. The details of retinoblastoma patients collected from Eye Hospitals in Coimbatore city, revealed that both sexes are commonly affected by this disease. Blood samples were collected from 58 retinoblastoma cases for the analysis of chromosomal aberrations using G-banding and the final results were ensured by multicolor spectral karyotyping (SKY). Chromosomal analysis of the peripheral blood cells in retinoblastoma patients showed that the chromosomes 1, 3, 5, 6, 8, 10, 11, 13 and 17 carry aberrations. The more frequent aberrations were 13q-, 6p+ and 1q-. Deletion in the 13q is considered as a pre-requisite for tumorigenesis whereas the 6p and 1q are involved mainly in tumor progression. The less frequent aberrations were 3q-, 5p-, 17p-, 8q-, t(17p; 6p) and t(13q; 10q). They may not have a role in the tumorigenesis of retinoblastoma. Chromosomes under the group A, C and D are more frequently aberrated.

Hence the above described chromosomal aberrations can be used as a marker to diagnose retinoblastoma patients. Our results revealed that conventional cytogenetics remains an important tool in elucidating the complex and diverse genetic anomalies of Retinoblastoma. According to these results, chromosomal analysis is of great use in patients with Retinoblastoma at diagnosis to have a correct prognostic evaluation for clinical decision making.

P03.099** Identification of copy number variations in early infantile onset Rett Syndrome variants

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Background: Early infantile onset Rett syndrome (RTT) is characterised by Rett syndrome-like presentation with early onset (before the age of six months).

Methods: Copy-number variations (CNVs) have been reported in children with mental retardation. In this study, we analysed 12 early infantile onset RTT patients in order to find potential genetic abnormalities in this particular group, using a high-resolution GeneChip SNP array technique.

Results: We found four deletions on the X chromosome; size rang-

ing from 1.1 Mb to 4.2 Mb (located on Xp11.22, Xp22, Xq22-q23 and Xq27). These regions are known to contain a number of important genes which are expressed in the brain and perform critical roles in neurite development, the initiation of process formation and axonal guidance in the developing neurons. The findings in this study imply that CNVs might be involved in this specific type of RTT (4/12 patients) rather than MECP2, CDKL5 or FOXG1 genes, since no pathogenic mutations were detected in any of these genes.

P03.100 Detection and Analysis of Serotonin Receptor Gene (5-HT3A) Expression on Human Peripheral Blood Lymphocytes in Rheumatoid Arthritis (RA) Patients

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Background and aim: The 5-HT3A receptor is a pentameric ligand-gated cation channel located in the central and peripheral nervous system and on extraneuronal locations like lymphocytes, monocytes and fetal tissue. The expression of the serotonin receptors was investigated in the brain but little work has been done to examine their expression in other tissues. The aim of this study was to show whether the serotonin receptor gene expresses on peripheral blood lymphocytes in the patients suffering from rheumatoid arthritis (RA) disease.

Material and methods: In the present study, using RT-PCR technique, we investigated 5-HT3A receptor gene expression in peripheral blood lymphocyte cells (PBMC) of forty RA patients.

Results: The results showed that the 5-HT3A receptor gene is detected on the lymphocytes of RA patients.

Discussion: There is not any evidence which points to role of serotonin and their receptor in the pathophysiology of RA. Therefore, we detected the expression of 5-HT3A receptor in patient individuals. Expression profile in RA patients was compared to healthy individuals. It can contribute to access some new information related to pathogenesis of RA disease.

P03.101 Ring 20 syndrome mosaicism: a new case with amplification of two BAC clones at 20q11.21-q22 without deletion of the telomeric regions, detected by the Genome Array-CGH.

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Ring 20 syndrome is a rare disease characterized by drug-resistant epilepsy, moderate mental retardation and particular electroencephalographic disorder with non-convulsive status epilepticus. Herein, we report on a new case of r(20) syndrome mosaicism in a 15 year old female that presented minimal dysmorphism, generalized tonic and clonic absence and seizures, refractory to medical therapy, and behavioural disorders. She was the first child of an unrelated couple, and she was born after an uneventful pregnancy and delivery. Among 50 analyzed cells, 30 showed a 46, XX karyotype and 20 exhibited a 46, XX r(20)(p13q13.3)(40%). FISH using whole specific probe of chromosome 20 confirmed the presence of the ring. Array-CGH (platform Bluegnoe Cytochips, Technogenetics) showed no deletion at the site of the ring formation, but found the presence of an amplification of two BAC clones, approximately 1.5 Mb, in 20q11.21-q22. We think that the lack of detection of any deletion could be attributed to the mosaicism and that clinical symptoms may be assigned either to haplo-insufficiency of two epilepsy genes, CHRNA4 and KCNQ2, which map in this region, or that other mechanisms may be involved. To the best of our knowledge, our patient was the first case presenting the typical epilepsy disorder but no detectable deletion in the ring chromosome 20 associated with amplification in 20q11.21-q22. Further studies are in progress to better genotype/phenotype correlation and to clarify whether there is any association of this particular rearrangement with

this characteristic type of epilepsy, occurring exclusively in patients with ring 20 syndrome.

P03.102 Developmental delay and autistic features in a girl with 45,X/46,X,r(X)

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We report here a case of a 2 years old female with developmental delay (sit unsupported at 11 months, walking independently at 18 months, no 2 words phrases by 25 months) and autistic features (limited communication, obsessive behaviour and social difficulties...). No lymphedema, neck webbing, congenital heart defects, dysplastic nails, nor facial dysmorphism were present.

Cytogenetics analysis of 48-GTG band metaphases (400-550 band) from peripheral blood lymphocytes prepared according to standard procedures revealed a 45,X karyotype in 24 cells and 46 chromosomes with absence of one X chromosome and the presence of a small ring chromosome in 24 cells. Fluorescence in situ hybridization (FISH) studies were also performed. Metaphase arm-specific chromosome painting probes, XCAPX long (Texas Red) and XCAPX short (FITC), revealed the presence of two red and two green signals, which is consistent with the fact that the ring is completely derived from X-chromosome material. With the XCyeX probe we confirmed that the r(X) had more than pericentromeric material (p11.1-q11.1). The presence of XIST (Xq13.2) was confirmed on both, ring and normal X chromosome.

Individuals with 45,X/46,X,r(X) cytogenetic constitution comprises about 6-7% of cases with a diagnosis of Turner syndrome. It has been noted that there is an increased prevalence in mental retardation and another atypical physical abnormalities in this group, in comparison with 45,X cases. The etiology of mental retardation and congenital malformation remains uncertain. Even in the presence of XIST expression, cases with abnormal phenotypes have been described. X inactivation may be incomplete despite XIST expression.

P03.103 MECHANISMS OF RING CHROMOSOME FORMATION

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We defined the breakpoints and mechanisms of ring chromosome formation in 12 patients using array-SNP Affymetrix® Nsp/Sty 6.0 and Illumina® platforms. The chromosomal terminal regions were investigated by MLPA and FISH techniques using subtelomeric and pantelomeric probes, respectively. The karyotypes of patients I-XII were determined, respectively: 46,XY,r(3)(p26.1q29), 46,XX,r(4)(p16.3q35.2), 46,XY,r(10)(p15.3q26.2), 46,XX,r(10)(p15.3q26.13), 46,XY,r(13)(p13q31.1), 46,XY,r(14)(p13q32.33), 46,XX,r(15)(p13q26.2), 46,XY,r(18)(p11.32q22.2), 46,XX,r(18)(p1.32q21.33), 46,XX,r(18)(p11.21q23), 46,XY,r(22)(p13q13.33) and 46,XX,r(22)(p12q13.2). These rings were found to have been formed by different mechanisms, such as: from breaks in both chromosome arms, followed by end-to-end reunion (patients IV, VII, VIII and XII); from a break in one chromosome arm followed by fusion with the subtelomeric region of the other (patients I-II); from a break in one chromosome arm followed by fusion with the opposite telomeric region (patients III and IX); from fusion of two subtelomeric regions (patient VI); from telomere-telomere fusion (patient XI). Thus, the r(14) and one r(22) can be considered complete rings, since there was no loss of relevant genetic material. The r(13) showed a more complex mechanism of formation resulting in a deletion of 11.1 Mb concomitant with an interstitial duplication of 1.5 Mb. His karyotype was 46,XY,r(13)(p13q33.1).arr13q33.1(101,543,509-103,001,462)x3,13q33.1q34(103,003,268-114,142,980)x1 dn. We concluded that the phenotypes of ring patients can be related with different factors, including gene haploinsufficiency, gene duplications and ring instability. Epigenetic factors due to the circular architecture of the ring chromosome must also be considered,

since even patients with complete ring chromosomes can present associated phenotypic alterations, as observed in our patients with complete r(14) and r(22). (Financial Support: FAPESP/Brazil).

P03.104 Ring chromosomes 9 and 22 in children

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Ring chromosome is a rare genetic disorder observed in the children with variable clinical presentation and phenotype. Constitutional ring chromosome occurs in 1:50000 live birth.

We describe four patients, one with ring chromosome 9 and three children with ring chromosome 22. Chromosomal analysis was performed using G banding according to standard procedure on peripheral lymphocytes.

Ring chromosome 9 is chromosomal aberration include growth and psychomotoric retardation, heart malformation, ambiguous genitalia, limb and skeletal defects. Cytogenetic analysis was found in all cells ring chromosome 9. After FISH analysis karyotype was 46,XY,r(9).ish r(9)(wcp+,D9Z1+). Parental karyotypes were normal.

Ring chromosome 22 is a rare occurrence. Most cases of r(22) would be expected to show loss of part or all of the short arm in addition to loss of some long arm material. Loss of short arm material is unlikely to have any phenotypic effect. Patients with ring chromosome 22 may have clinically significant deletions of 22q and application of FISH techniques to ring chromosomes in order to confirm that deletion has occurred, is a useful adjunct to conventional cytogenetic studies. However, partial monosomy resulting from deletion of part of the long arm is known to be associated with a syndrome involving global developmental delay, mental retardation, severe delay in speech and language skills but only minor facial dysmorphology.

All patients were informed by genetic counsellor and they are included in multidisciplinair programs.

P03.105 Molecular and clinical definition of an unstable ring chromosome 21

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Constitutional ring chromosomes are generally believed to be the result of de novo breakage of both end-segments of a chromosome during meiosis or early postzygotic mitosis, followed by ends joining, resulting in a continuous ring. This mechanism presumes the loss of some genetic material during ring formation. Almost one-half of reported ring autosomes derives from acrocentric chromosomes. However ring 21 [r(21)] is a rare finding. Here we report a case of r(21) showed to be unstable by SNP array. The proband is a 6-year-old boy with mental retardation, epilepsy, facial dysmorphism, myopia, and equinovarus clubfeet. Brain MRI showed leucoencephalopathy. Karyotype and FISH analysis revealed a 46,XY,r(21)(p11q22).ish r(21)(p11q22)(wc p21+,tel21q-,RP11-61A21+,RUNX1+,LSI21-) formula. Accurate molecular characterization by SNP array (Illumina) and BACs-on-Beads technology (BoBs) confirmed a 21q22.2 deletion and showed a mosaic proximal 21q deletion. We suggest that ring instability and death of aneuploid cells may explain the microarray results. To confirm this hypothesis, FISH analysis on uncultured lymphocytes is programmed.

P03.106 Ring Chromosome 13: Characterization by molecular cytogenetic and DNA Micro-array analyses.

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Ring Chromosome 13 is a rare chromosomal abnormality in mental retardation. We report three cases of ring chromosome 13. In the first

case a female with a karyotype: 46, XX, r (13) [17]/ 45, XX,-13 [33], she revealed a language retardation and dysmorphic feature. Patient 2 is a boy with a karyotype: 46, XY, r (13) [13]/ 45, XY,-13 [3] dead in 11 years old by leukemia. Finally the third case is a boy with congenital cardiopathy and mental retardation his karyotype showed 46, XY, r (13). For further characterization of the ring chromosome 13, FISH was performed on metaphase chromosomes from the patient's cells using three DNA probes: WCP13, 13qter and LIS13 (13q14)/RB-1(Vysis®). Both cases showed loss of the 13q subtelomeric region by fluorescence in situ hybridization and chromosome whole painting probe showing a homogeneous painting of the whole normal and ring chromosomes 13 for the three patients. FISH analysis using LIS13(13q14)/RB-1 probes showed a deletion of RB1 gene only in the ring (13) of patient 2. For the first patient Agilent® oligonucleotide arrays 44K were performed for more characterization of the ring 13 chromosome. It showed a deletion of 4, 7 Mb in 13q34 region and a deletion of 2, 9 Mb in 13q11q11 region. Molecular cytogenetic and whole-genome array-based technologies can give an accurate delineation of the imbalances which raises the possibility of making genotype-phenotype correlations, in order to identify minimal critical regions and candidate genes for a pattern of clinical features including learning disability

P03.107 Prenatal Diagnosis of de novo 44,XX,der(13;14)(q10;q10)/46,XXX,der(13;14)(q10;q10) Karyotype

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Robertsonian translocations represent the most common human structural chromosomal abnormality, occurring with an incidence of 1 / 1,000 in the general population. The occurrence of X chromosome monosomy and Robertsonian translocation in the same person is rare. Turner Syndrome is a chromosome abnormality affecting about 1 in every 5000-10000 female births and up to 20% of miscarriages. Many, perhaps most, girls and women with Turner Syndrome are actually mosaic, meaning that they have cells with more than one karyotype. Often there is a mix of 46,XX and 45,X cells, and in this case the resulting clinical abnormalities tend to be milder than in 45,X Turner Syndrome. We report a fetus diagnosed prenatally with both a Robertsonian translocation and mosaic Turner's syndrome. Amniotic fluid sample was taken from our patient at the 20 weeks of pregnancy, due to an increased second trimester screening risk. Chromosome analysis was performed on trypsin G banded chromosome preparations obtained from amniotic fluid cultures. The karyotype was designed as 44,X,der(13;14)(q10;q10)[23]/46,XXX,der(13;14)(q10;q10)[16] Peripheral blood samples were taken from parents which revealed normal karyotypes. The family was informed during the genetic counseling session and they decided on termination of the pregnancy.

P03.108 An Association studies for search a Susceptibility Genes in Schizophrenia

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Objective: Schizophrenia is a severe mental disorder affecting 0.4-1% of the population worldwide. It is characterized by impairments in the perception of reality and by significant social or occupational dysfunction. The disorder is one of the major contributors to the global burden of diseases. Studies of twins, families, and adopted children point to strong genetic components for schizophrenia, but environmental factors also play a role in the pathogenesis of disease.

Materials and Methods: Molecular genetic studies have identified several potential positional candidate genes. The strongest evidence for putative schizophrenia susceptibility loci relates to the genes encoding dysbindin (DTNBP1) and neuregulin (NRG1), but studies lack impressive consistency in the precise genetic regions and alleles implicated. Results: The present study shows the role of three potential candidate genes by genotyping in the DTNBP1, NRG1 and AKT1 genes in schizophrenia patients. Some previously identified a region on chromosome 5q21-34 as a susceptibility locus for schizophrenia. Recently, two studies reported association between the g-aminobutyric acid type A receptor cluster of genes in this region and one study showed suggestive evidence for association with another regional gene.

Conclusion: This is the first candidate gene study to explicitly test for and provide evidence of a maternal-fetal genotype incompatibility mechanism in schizophrenia. In the present study found evidence that one GABA receptor subunit, GABRG2, is significantly associated with schizophrenia. Furthermore, it also seems to affect to the functioning of the working memory.

P03.109 A simple and rapid method for heterochromatin regions selective staining on direct chromosomal preparations from tissues with spontaneous mitotic activity.

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Allknown techniques for selective stainig of polymorphic pericentromeric heterocromatin regions and Yqh in human cromosomes include CBG, DA/DAPI, MG/DAPI, D278/170, MG/H33258. Acridine orange (AO) is routinely used in for RFA and RBA banding. Our study was carried out on direct and semi-direct chromosome preparations from human chorionic villi and from different embryonic samples at the 5-12 weeks of gestation (more 200 samples). After AO staining without any pretreatments the constitutive heterochromatin regions (CHRs) in 1qh, 3cenh, 4cenh, 9qh, 16qh, 15cenh, 22cenh and Yqh showed red fluorescence, whereas all chromosome arms were uniformly yellow-green. This staining pattern was irrespective of AO concentration (0.01-0.1%), buffer solution (PBS, Sorensen's buffer, McIlvain's buffer), its pH (6.5-7.2) and age of slides (from 1 day to some months). The red AO fluorescence of CHRs was detected also on metaphase chromosomes of bone marrow cells from patients with lymphoblastic leukemia (10 samples were kindly provided by Dr. I.Martynkevich). While they stained in green in cultured amniocytes (4 samples were kindly provided by Dr.Y.Loginova). CHRs from PHA-stimulated lymphocytes (more 50 samples of fetal and adult origin) revealed yellow-green staining in most metaphases with red 9qh in some metaphases. Mechanism of this selective staining remains unclear. However, as AO is metachromatic stain which discriminates single-stranded from double stranded nuclei acids, our results advocate in favor of the principal cytochemical and conformational differences in CHRs in different cells which could be attributed to the variability of their DNA and histone epigenetic modifications.

P03.110 Chromosome abnormalities in girls with isolated short stature

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Objective: Short stature is defined as a condition in which the height of an individual is more than 2 SD below the corresponding mean height for a given age, sex and population group. Many different genetic etiologies of short stature are known. The present study aims to evaluate whether isolated short stature (SS) is an adequate indication for routine chromosome analysis.

Materials & Methods: This is a retrospective survey of 371 girls who were referred for cytogenetic investigation at the Department of Medical Genetics of Athens University between the years 1999 - 2009 for SS only. Girls who in addition of SS had other phenotypic features were excluded. The mean age of referred subjects was 9.5 years. Karyotyping was performed on cultured peripheral lymphocytes using GTG-banding.

Results: Abnormal karyotype was detected in 34 (9.2%) of the referred cases. Approximately 20% of the girls with abnormal cytogenetic findings had a 45,X0 karyotype (n=7), while the remaining 80% were mosaic for Turner syndrome with 2-4 cells lines (n=27). Four girls (1.1%) carried chromosome variants (3 inv(9) and 1 inv(6)) and one individual had a balanced translocation which did not involve the sex chromosomes.

Conclusion: Cytogenetic investigation is essential in the management of girls with isolated SS to exclude Turner syndrome, in which effective, but costly, growth hormone treatment has shown to improve final height.

P03.111 Combined direct dup(7)(q22q33) and inv(7)(q32.3q31.2) in a developmentally delayed and dysmorphic boy

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De novo inverted duplications are very rare chromosomal aberrations. Chromosome 7 duplications have been described in patients with Silver-Russell syndrome indicating possible relevance for this etiologically heterogeneous syndrome. We report on a male patient who shows muscular hypotonia, global developmental delay, and various dysmorphisms resembling Silver-Russell syndrome. Both parents (31-year-old mother and 29-year-old father) and two older sibs are healthy. Family history as well as pregnancy and delivery were unremarkable. Length (52 cm), weight (4.030 g), and occipitofrontal head circumference (37 cm) at birth were around the 90th percentile. At the age of 5 years height (101.5 cm) was at the 3rd percentile, weight (17.3 kg) was at the 10th percentile, and occipitofrontal head circumference (51 cm) was at the 25th percentile.

Conventional karyotyping and FISH analysis with YACs revealed a 46,XY,dup(7)(q22q32)inv(7) (q32.3q31.2) karyotype. SNP microarray analysis with the Illumina® Infinium HD HumanOmni1-Quad v1.0 BeadChip defined the proximal and the distal breakpoint of the entire duplication between rs10275844 and rs6960639 and rs10954272 and rs6467310, respectively, which includes the intrauterine growth retardation- and Silver-Russell syndrome-related imprinted region on 7q32. The duplicated region has a size of about 31.7 Mb. Investigations for the parental origin of the rearrangement as well as fine-mapping of the breakpoints of the inversion and the duplication are in progress.

The constellation in our patient is another example for the observation that apparently simple chromosomal rearrangements may be more complex than initially thought, and that duplication of 7q32 may cause at least some clinical features of Silver-Russell syndrome.

P03.112 Skewed X chromosome inactivation is associated with OTC deficiency in 9-year-old girl with karyotype 46,X,del(X)(p11)

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Ornithine transcarbamylase (OTC) deficiency, the inherited urea cycle disorder, is transmitted as a partially dominant X-linked trait (mapped to band Xp21.1). The symptoms in females vary both in onset and severity. Some of them are completely asymptomatic, while others have severe hyperammonemic crises, which can lead to brain damage or even to death. X-inactivation occurs very early in embryogenesis. It is usually a random process and both paternal and maternal alleles are randomly inactivated. In some cases, the X-inactivation is skewed. When the inactivation is skewed to express more of the mutated allele, X-linked disorders may affect female carriers in different ways.

We report a 9-year-old girl who had problems since the birth (respiratory insufficiency, hypoglycaemia), later developmental delay, muscular hypotonia, seizures, hepatomegaly and frequent infections. Metabolic investigations showed periodic hyperammonaemias. At 3-year of age OTC deficiency was diagnosed based on allopurinol loading test results (increased orotic acid excretion). Despite the treatment (protein restriction, L-citrulline and anticonvulsants) she had recurrent seizures which were controlled only with sodium-phenylbutyrate. At the same time ammonia was normal. Standard G-banding cytogenetic and whole genome array analysis showed abnormal karyotype 46,X,del(X)(p11). The X-inactivation study showed that the X inactivation was skewed: in 14% of cells the aberrant X-chromosome was active. It is previously known that in cases where the breakpoint in Xp is proximal to Xp22.3, there is a skewed X-inactivation, with the aberrant X being preferentially inactivated. In our case we saw unusual X-inactivation which is responsible for the atypical course of OTC deficiency.

P03.113 A possible association between the SLC9A gene and epilepsy - a case story

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The SLC9A9-gene (NHE9) belongs to a family of Na+/H+exchanger membrane proteins, which regulates ion fluxes across membranes. The SLC9A9 gene has in several studies been associated with language delay, ADHD and autism. Other genes from the Na+/H+exchanger family have been associated with epilepsy in mice. A possible association to epilepsy in humans was shown by Morrow et al., 2008, who described a family of a mother and 2 sons with a non-sense mutation in the SLC9A9 gene. Both sons had autism, and one was diagnosed with epilepsy, while the other had had some seizures. Their mother had speech delay, but neither autism nor epilepsy.

We describe a mother and son with a 0,79 MB duplication of chromosome 3q23-q24 including the SLC9A9 gene, diagnosed by Array CGH. The woman had epilepsy since the age of 11 years. She was treated with Valproate 3000 mg daily during pregnancy. Her son was born with a phenotype compatible with Foetal Valproate Syndrome, and in addition language delay and some behavioural problems. At the age of four years, he did not have signs of autism, ADHD, or seizures.

Conclusion: We describe a mother and son with duplication of the SLC9A9-gene. The mother was diagnosed with epilepsy at the age of 11 years. The son had Foetal Valproate Syndrome, and had no history of seizures at the age of four years. This case supports an association between the SLC9A9 gene and epilepsy in humans. However more studies are needed in this area.

P03.114 A new case of 4pter duplication and 4qter deletion detected by SNPs-array.

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Only one case of duplication of the distal part of 4p and deletion of the same part of 4q as been so far described. This condition give rise to recognizable craniofacial features: prominent glabella, characteristic bulbous nose, retrognathia, pointed chin, short neck with low hairline, enlarged and abnormal ears and cardiac defects. Our patient is 9-years-old boy mentally retarded, born from non consanguineous and healthy parents. He was born at 34 weeks, with a birth weight of 2.140g (<3%). His clinical history was characterized by: interatrial defect (involved spontaneously), frequent otitis and bronchitis and right hernia (he underwent surgical treatment at 3 years old). He showed facial dysmorphic features as: large forehead, downslanting palpebral fissures, telecanthus, prominent glabella, flat nasal root, pug nose with anteverted nares, long philtrum, thin upper lip, malformed ears, short neck, brachydactyly. The karyotype was 46 XY normal. SNPs-Array (370K from Illumina) showed a 4p15.3p16.3 terminal duplication (14.9 Mb) and a 4q34.2q35.2 terminal deletion (14 Mb). Our patient overlap clinical history, cardiac defects and dysmorphic features of the previously described case. The deleted region is bigger in our case than in the previous one. Anyway, our patient confirms that cardiac defect are probably due to the deletion of ArgBP2 and PDLIM3 genes in 4q region. We agree that the duplicated region may essentially facial dysmorphisms, also present in cases affected by a 4p trisomy. In conclusion, we might suggest that both these cases with their clinical pictures define a new syndrome of contiguous genes alterations.

P03.115 Familial mental retardation: role of subtelomeric translocation

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Introduction: The identification of subtelomeric rearrangements as a cause of mental retardation (MR) has made a considerable contribution to diagnose MR.

Aim: To correlate genotype/phenotype in 1p subtelomere deletion and 16p subtelomere duplication. Diagnosis of the balanced carriers for prevention of recurrence of MR

Methods: This study carried out on two children who have mental retardation (MR) and dysmorphic features. They are members of a large family with recurrence of mental retardation. Cytogenetics and total

multi color FISH were performed.

Results: Pedigree analysis revealed fourteen MR cases and eight obligate carriers in four generations. Both probands have MR, dysmorphic features and cleft palate. Conventional cytogenetic analysis showed normal karyotype in the two probands and their parents. Total subtelomeric multicolor FISH in the 1st proband revealed ish der(1)(1;16)(p36.3,-p13.3+), his father has balanced translocation ish t(1;16)(p36.3,-p13.3+;p13.3,-p36.3+). The 2nd proband has the same unbalanced subtelomere translocation and his father has the same balanced translocation.

Conclusion: The pedigree of this large family indicates that the Subtelomere translocation does not affect fertility. This carries a high risk of transmission of MR to several generations. In such family genetic testing of all members is a must. Prevention of recurrence of MR can be achieved through carrier detection and PGD.

P03.116 Identification and interpretation of supernumerary marker chromosomes

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Supernumerary marker chromosomes (SMCs) are extra, abnormal chromosomes whose origin cannot typically be determined by conventional chromosome-banding techniques. SMCs are common, occurring in four of every 10,000 newborns, but are approximately 7 times more prevalent in individuals with mental retardation. The most common class of marker chromosomes are derived from acrocentric chromosomes and have a satellites or bisatellites structure, with chromosome 15 accounting for the highest percentage of this group.

We present two cases of SMCs. The first one is a prenatally diagnosed partial mosaic trisomy of chromosome 22 with the karyotype 46,XY[65]/47,XY,+mar[35]. Fluorescent *in situ* hybridization (FISH) and spectral karyotyping (SKY) techniques identified a SMC as an inv dup(22)(q11.1) consists of only bisatellites heterochromatic material. In the literature there are described several cytogenetically similar cases, which did not show any clinical abnormalities. In the second case we report a girl with craniofacial dysmorphism and multiple anomalies consistent with the supernumerary der(22) syndrome. Derivative 22 [der(22)] syndrome is rare disorder associated with multiple congenital anomalies including pre-auricular skin tags or pits, conotruncal heart defects, and profound mental retardation. The nature of the SMC was characterized using FISH and SKY techniques as der(22)t(11;22)(q23;q12). The karyotype of her mother showed a reciprocal translocation over the distal bands 11q23 and 22q12.

SMCs are a major clinical problem, especially when detected prenatally during cytogenetic analysis. Molecular cytogenetic techniques are necessary for their comprehensive characterization. In general, the risk for an abnormal phenotype in prenatally ascertained *de novo* cases with SMCs is given as ~13%.

P03.117 Identification of microdeletion 1q21 by FISH and array-CGH in proband with TAR syndrome

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Thrombocytopenia-absent radii (TAR) is a rare genetic disorder. In patients with TAR syndrome microdeletion on chromosome 1 (1q21.1) was detected, but the presence of an additional modifier is required to elicit the phenotype. It was observed maternal as well as paternal transmission and occurrence *de novo* in 25 %.

We performed molecular-genetic methods to detect range of deletion in proband with TAR syndrome.

In proband was ascertained bilateral radial aplasia, annulled ulna and scaffold of hands normally formalized. Ultrasound of brain was without pathology. Cardiology examination found foramen ovale apertum covered by aneurismatic lash camber. Investigation of the bone marrow proved deficit of megakaryopoiesis and nonspecific dysplastic changes. TAR syndrome was determined as a clinical diagnosis.

We determined presence of microdeletion by fluorescence *in situ* hybridization (FISH) analysis using RP11-311I20 (BlueGnome) probe and as a control probe we used centromere probe of chromosome 1 (Abbott Vysis). They were hybridized to metaphase spreads of the

patient's lymphocytes after PHA stimulated cultivation of peripheral blood by use of standard procedures. Oligonucleotide array-CGH (Human Genome CGH Microarray Kit 44K, Agilent Technologies, USA) was used for deletion sizing.

The FISH results revealed deletion of RP11-315I20 probe on one chromosome 1 in locus 1q21.1 in proband. aCGH confirmed this deletion. The deletion had a maximum size 350 Kb and encompasses 21 oligonucleotides. Both parents of the proband were also investigated by FISH. The deletion of RP11-315I20 probe was proved in mother. FISH and array-CGH are suitable methods for detection chromosome aberrations in rare clinical syndromes.

P03.118 A case of de novo partial trisomy of short arm of the chromosome 4.

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We report newborn male with multiple congenital abnormality: hypotrophy(m=1840g), asymmetry of the face, depressed nasal bridge, bulbous nasal tip, macroglossia, low set and malformed ears, short neck, abnormal dermatoglyphics findings, polycystic kidneys, small penis and cryptorchism. An additional material of unknown origin was detected on distal part of long arm (q) of the chromosome 10 in blood lymphocytes culture analized with GTG- banding. Thus the proband's karyotype was 46,XY,add(10q). Parents' karyotypes were normal. For detection of further detailed analysis FISH with different DNA-probs was applied: WCP 4/10, subtel 4 p/q (Vysis, Abbott, USA), mFISH (24XCyte Vit (MetaSystems, Germany)), a result the proband karyotype was 46,XY,der10t(4;10)(p14;q26).ish der(10)(wcp4p+);dn. In finally proband had the trisomy 4p and monosomy 10(q26).

P03.119 Particular phenotype in a newborn with partial trisomy 10p and partial monosomy 9q

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Trisomy 10p is a rare chromosomal syndrome. Most of the reported trisomy 10p cases represent unbalanced products of familial reciprocal translocations and are complicated by additional segmental imbalances. We present a female newborn with severe facial dysmorphies and other anomalies, due to trisomy 10p and telomeric deletion of 9q. The pregnancy was followed, and ultrasound examination revealed an increased nuchal translucency, but the mother refused other examinations. The newborn, delivered at term presented: growth retardation, cranio-facial dysmorphies (microcephaly, high forehead with hirsutism, hypertelorism, short palpebral fissures, anteverted, hypoplastic nostrils, thin lips, macroglossia, low-set, folded ears), supernumerary nipples, flexed fingers, plantar creases, hypotonia, tetralogy of Fallot and multicystic left kidney. Cytogenetic analysis from peripheral blood lymphocytes showed an extra material on the long arm of chromosome 9 (46,XX,add(9)(q34)). The mother's karyotype revealed a translocation (9;10). FISH analysis was performed for the mother, using 9q telomere and 9p telomere probes, and the telomere 9q signal of the derivative chromosome was located on the short arm of abnormal 10 chromosome. FISH using telomere 10p and 9q probes was performed for the proband and revealed partial trisomy 10p and partial monosomy 9q. The evolution of our patient was unfavorable, as she died two weeks after birth. Comparing our case to trisomy 10p cases and telomere 9q deletion cases the proband associated unlike the reviewed reported cases, supernumerary nipples. For best evaluation, arrayCGH should have been performed, but it is no longer possible as the newborn died and no DNA sample was taken.

P03.120 Molecular karyotyping of prenatal samples using oligonucleotide-based ArrayCGH: A technical feasibility study

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Objectives: Karyotyping of banded metaphase chromosomes is currently the gold standard for the detection of chromosomal aberrations in prenatal samples. The present technical feasibility study aimed at comparing results of conventional karyotyping and oligonucleotide ar-

rayCGH in prenatal samples and association of the laboratory findings with clinical features.

Methods: DNA was extracted from prenatal (CVS) samples with known karyotypes left over from conventional genetic analysis and fully anonymised. The series was enriched for cases with chromosomal aberrations (miscarriages). Array CGH was performed on NimbleGen CGX-12 arrays. This array contains 135,000 oligos covering more than 200 targeted regions and more than 675 (functionally significant) genes plus 41 unique sub-telomeric regions and 43 unique peri-centromeric regions. All of these regions have a coverage of one probe every 10 kb. The whole-genome backbone coverage is one oligo every 35 kb

Results: DNA of sufficient quality and quantity can be routinely obtained from CVS samples for performing array CGH. Protocols for labeling and hybridization were robust. The laboratory process from extracting DNA to finishing the array scanning took usually less than 3 days, and, thus, was significantly shorter than the time frame for long-term culturing. Though in depth analysis of the array CGH results along with comparison to clinical features is ongoing, initial findings suggest that clinically relevant aberrations are readily detectable by molecular karyotyping.

Conclusions: ArrayCGH might supplement karyotyping of CVS samples particularly in cases with structural chromosomal aberrations detected by conventional chromosome analyses or aberrant first trimester screening.

P03.121 Unusual signs in a phenotype of a patient with trisomy 8 mosaicism

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Mosaic trisomy 8 is a relatively common chromosomal abnormality, with a distinct, recognizable phenotype in most cases. We report a case, which in addition to certain characteristic features of mosaic trisomy 8: a long, slender trunk, limitation of movement in multiple joints, and mild-to-moderate mental retardation, the deep plantar furrows, presented several signs including craniofacial midline defects: notched nasal tip, cleft maxillary alveolar ridge, bifid tip of tongue, grooved uvula and left choanal atresia. Transfontanellar ultrasound revealed absence of corpus callosum and enlargement of the occipital horns of lateral ventricles. Cardiac ultrasonography showed an atrial septal defect. Due to the presence of midline defects, we suspicion the possibility of Toriello-Carey syndrome.

Chromosome analysis on peripheral blood lymphocyte culture revealed trisomy 8 in 12 metaphases out of a total 50 examined metaphases. FISH analysis on cultured blood lymphocytes with CEP of chromosome 8 showed 3 hybridization signals in 25 % of cells.

Knowing that even it is a rarely possibility, the association of two syndromes may appear, an arrayCGH was performed to investigate the possibility of cryptic unbalanced rearrangements. The commercially available array (GenoSensor Array 300, Abbott) containing 287 genomic targets did not detect genomic imbalance.

We described this case of mosaic trisomy 8, which in addition to certain characteristic features of this syndrome, presented craniofacial midline defects, because these were not previously described in this chromosomal disorder. We consider that the clinical findings in our patient may contribute to the extension of the phenotypic spectrum of trisomy 8 syndrome.

P03.122 A report of a case with partial trisomy 9(p22pter)

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We present a 7 year -old girl who referred to our laboratory for cytogenetic investigation. The girl had frontal bossing, left bridge protrusion, and left upper lip down, open mouth. She was hyperactive and developmentally and mentally delayed. She had speech delay. The cytogenetic test performed using standard method. The karyotype of the proband showed an additional material at 4q.

The mother karyotype confirmed that the additional segment on 4q was of chromosome 9p origin, resulting in trisomy 9(p22pter). The karyotype of the proband was found as:46,XX,der(4)t(4;9)(~35.2q;22p). Her father's karyotype was 46, XY.

The parents advice to seek genetic counseling and informed of the

possibility of having further child(ren) with partial trisomy or monosomy of the relevant chromosomes.

P03.123 Mild Turner Syndrome caused by Xpter-p21.1 duplication and Xq22.3-qter deletion

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The classic phenotype of Turner syndrome includes short stature, ovarian failure and variable somatic stigmata. It is associated with X monosomy. Mosaic and partial deletions or duplications of chromosome X result in different degrees of Turner syndrome. Few cases have more complex chromosomal complements. Here we report a woman with mild Turner syndrome caused by partial duplication of Xp and deletion of Xq.

A 19-year-old woman was first time investigated due to primary amenorrhea, infantile external genitals and hypergonadotropic hypogonadism. Her growth was normal 172 cm (+1SD), BMI 16.9. She had some dysmorphic features: a low hairline, clinodactyly, and joint laxity. Chromosomal analyses revealed a female karyotype with derivative X-chromosome (Xq-). FISH-analysis with wcpX and XYpter and XYqter subtelomeric probes (Cytocell) showed, that chromosome consisted entirely of X-chromosome material and both arms had short arm signals on subtelomeric regions. Her parents had normal karyotypes (routine analysis and subtelomeric FISH-analysis). Further, using whole-genome Infinium-2 array and HumanCytoSNP array, we specified, that the patient has a 31.4-Mb duplication in region Xp22.33-p21.2 and 48.4-Mb deletion in region Xq22.3-q28.

Formation of der(X) chromosomes in females can be more complex than previously thought. In our patient, partial Xp trisomy and partial Xq monosomy cause mild Turner syndrome. The normal height of our patient can be explained by a trisomy of the Xp-located *SHOX* gene. However, she had amenorrhea and hypergonadotropic hypogonadism, which is common in Xq22-qter deletion patients.

P03.124 Mapping of the common deleted region of chromosome 7 in Uterine Leiomyoma through array CGH analysis

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Uterine leiomyomata (UL) are the most common benign neoplasm present in women of reproductive age, with approximately 40% of tumors showing structural cytogenetic abnormalities. Cytogenetic analysis has been fundamental in gene discovery efforts in these tumors. One of the most common recurrent abnormalities in UL involves deletion or rearrangement of the long arm of chromosome 7; however, the underlying genetic mechanism of this frequent aberration remains unknown. Identification of the pathogenetic sequence has been complicated by difficulty in determining the smallest deleted region. With this study, we sought to determine whether a common gene/region is involved in UL with translocation of chromosome 7 in band q22.

Using the human Agilent 1M array to assess genomic variation, we analyzed four UL with translocations of the long arm of chromosome 7. These rearrangements include: t(7;9)(q22;q22), t(1;7)(q42;q22), t(7;12)(q22-31;q14) and t(4;7)(p15;q22). Array-comparative genomic hybridization revealed accompanying large deletions in three of the tumors with a common loss of ~16.3 Mb within bands 7q21.11 and 7q22.1. Comparison of findings of this study with previously published data from our group resulted in a shortest region of overlap of ~3.3 Mb, restricting the possible candidate genes present in the region to eight genes: ZNF498, ZKSCAN1, TRIM4, ZSCAN21, ZNF655, C7orf47, COPS6 and PTCD1. These candidate genes will be further investigated to evaluate their possible role in UL development.

P03.125 Williams Beuren critical region duplication due to a supernumerary marker in a patient with autism and severe behavioural disturbance

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We report on a 3 years old boy with a mosaic 7q11 trisomy. The pregnancy was uneventful, birth measurements were within normal range and motor milestones were delayed (sitting achieved at 10 months and gait at 17 months). The language was markedly impaired (first word at 27 months). He presented with deep mental retardation and suffered from particular behavioural troubles consisting in the alternation of periods of apathy and aggressiveness. Social interactions were poor and so, autism was diagnosed. Facial dysmorphia was consistent with those reported in Williams Beuren Syndrome Critical Region (WBSCR) duplicated patients. Macrocephaly was also observed (+4SD). MRI showed non-specific posterior white substance anomaly and cardiac ultrasound was normal. Standard and molecular Karyotype (FISH and whole genome SNP genotyping array - Illumina), was mos 46,XY[50%]/47,XY,+mar[50%].ish 7q11.11q11.23(D7Z1+,ELN+).arr 7q11.11q11.23(62,000,000-74,000,000)x3dn. This 7q11.23 cytoband (12Mb) encompasses the WBSCR but also AUTS2, possibly involved in autism, CALCN1 which is expressed in brain and WBSCR17. Uniparental disomy of chromosome 7 was excluded.

This is the first report of WBSCR mosaic duplication due to a supernumerary marker. All patients reported so far carry the same 1.4-1.5Mb NAHR mediated duplication.

Our patient shared similarities with the patients already reported but presented also with several differences due to the size of duplication comprising genes expressed in brain and involved in autism. Thus, this observation underlies the involvement of these genes in this particular phenotype.

P03.126 Sibs recurrence of Wolf-Hirschhorn Syndrome resulting from a paternal cryptic translocation (4;15) characterized by FISH and SNP array analysis.

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Wolf-Hirschhorn syndrome (WHS) is a segmental aneuploidy caused by partial deletion of 4p. WHS is originated by different mechanisms including de novo 4p terminal or interstitial deletions or an unbalanced translocation (45%) either de novo or inherited from a familial balanced translocation (~15%). Clinical modifications in the phenotype could be explained by variation in the size of the deletion. We report two siblings with WHS due to a cryptic translocation (4;15)(p16.1;p11.2)pat. Both patients had similar clinical findings; however the girl also showed urinary tract anomalies. High resolution banding were performed and for FISH analysis LSI WHS region/CEP4 and mixture #4 (4p,4q,21q and LSI AML1) and #10 (10p,10q,15q and LSI PML) of ToTelVysion (Vysis) were used. SNP array was done using the GeneChip Human Mapping 500K platform (Affymetrix). Cytogenetic analysis in both children revealed a deletion of 4p, while both parents revealed an apparently normal karyotype. FISH analyses using LSI WHS region probe demonstrated in both sibs a submicroscopic deletion (4p16.3) while the father revealed a subtle balanced translocation involved a group D chromosome; to confirm that finding in the father a mixture #10 subtelomeric probe was used. A subtelomeric 4p region was observed in the boy and absent in his sister. SNP array confirmed different 4p interstitial deletions, being a ~6.31 Mb in the boy and 6.41Mb in the girl. Our results support the need of using FISH and high-density array for optimal characterization of the genetic imbalances in patients and will contribute to further correct phenotype/genotype correlations.

P03.127 X-inactivation silencing is not maintained on the autosomal segment of an inherited unbalanced X;19 translocation in a male**

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Turkey, ³Institute of Human Genetics and Anthropology, Jena, Germany. X-chromosome inactivation (XCI) is a programmed transcriptional silencing in mammalian female embryos. In naturally occurring X;autosome translocations, the inactivation signal spreads to the autosome and leads to delayed replication. Here we report the allele specific expression profiles of genes selected from the autosomal portion of the translocation in a unique male with 46,XY,der(19),t(X;19)(q11.1-11.2;p13.3) karyotype and bilateral periventricular nodular heterotopia, severe learning disability and epilepsy (Dev Med Child Neurol, 49: 219-24, 2007). The mother as well as the maternal grandmother of the patient is carrier of the same translocation, albeit in a balanced state with no phenotypical consequences. XCI analysis showed that selection pressure favors an active translocated X in the mother and the opposite in the patient. High resolution SNP genotyping array positioned the translocation breakpoints between the telomere and rs8105536 (212,033 bp) on chromosome 19, and between rs34355157 (62,794,454 bp) - rs3892372 (63,312,400 bp) on the X. This suggests a very small subtelomeric deletion (less than 0,21 Mb) of 19p13.3-19pter and a large duplication (almost 95,4 Mb) of Xq11.1-Xqter in the patient. Allelic expression analysis of 15 genes, including NDUFS7 at 19p13.3 and DNMT1 at 19p13.2, revealed that seven are heterozygous and are expressed biallelically in the patient. These results demonstrate that the transcriptional silencing of the genes on the autosomal segment of the translocation is not maintained, and that a compromise between stability versus plasticity occurs early in development.

P03.128 Association of breakage on Xq region and sterility

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According to Lyon hypothesis, in somatic cells of mammalian females, one X chromosome is randomly inactivated at the onset of embryonic development. The inactivated X is late replicating and can be cytogenetically distinguished from the active one. The analysis of balanced X;autosome translocations has revealed that in most cases the normal X is constantly late replicating.

A few published data showed that translocations between X chromosome and an autosome are very rare and are usually associated with primary amenorrhea and sterility.

Our proposita, aged 17, was referred to our department because of primary amenorrhea. She was the fourth child of a non-consanguineous parents. She has two healthy brothers and three healthy sisters. Her father was 47 and her mother 35 years old when she was born with the normal delivery. Her axillary and pubic hair was scant (Tanner 2), and her breast development was scaled Tanner 2. Ultrasonography showed a hypoplastic uterus and ovaries.

Investigation for chromosomal anomalies was routinely performed by cytogenetic analysis of our proposita and her parents. The karyotypes were carried out by G-banding technique studying 30 cells from the peripheral blood. Her karyotype was: 46,X,t(X;2)(q22;q23), and her father and her mother showed normal karyotypes.

It has been suggested that there is a critical region on the long arm of the X chromosome, ranging from Xq13 to Xq26, and breakage in this region would result in gonadal dysgenesis and sterility. In our proposita, the breakpoint was inside the critical region and caused sterility.

P03.129 The relationship of X;20 translocation and amenorrhea

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It has been suggested that translocations involving X chromosome and an autosome are rather rare due to the association with infertility in men and subfertility in women, being estimated to occur in about one in 30,000

live births. Some researchers showed that male carriers of an X-autosome translocation, which is either inherited from their mother or is de novo, are sterile, regardless of the position of the breakpoint in the X chromosome. A few published data revealed that in women, X-autosome translocations are frequently associated with primary or secondary ovarian failure.

We report a 23-year old woman who was referred to our department for chromosomal study due to secondary amenorrhea. Her parents were nonconsanguineous. She had three brothers and one sister, all

were older, healthy, and were married and had healthy children. Our proband had married six years ago, and claimed that before her marriage, she had regular menstrual periods, but after marriage, her periods became irregular by 2-3 months and stopped within a couple of years.

Her physical features including build, height, breast development, body hair and intelligence were normal.

Her pelvic sonography showed normal size and echo pattern of her uterus and ovaries. Evaluation of her hormones showed elevated for FSH and LH.

The G-banding technique was performed on her cultured peripheral blood, and revealed: 46,X,t(X;20)(q21;q13).

The presence of t(X;20) plus hypergonadotropic amenorrhea in our proband confirms the role of X-autosome translocations in ovarian failure.

P03.130 Karyotype/phenotype correlation in Egyptian patients with Xeroderma pigmentosum.

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Xeroderma pigmentosum is a very rare hereditary disorder. We studied 14 Egyptian patients clinically diagnosed as xeroderma pigmentosum (XP). The main aim was to determine the effect of karyotype variation on phenotype. Patients were clinically classified and the number of sister chromatid exchanges were calculated for each case. Karyotype studies showed the following results: 12 & 13 % SCEs were detected in 28.6% of patients, 8.8-11% in 57.1% of the patients whereas 7.9% and 5.9% in two patients respectively. Karyotype/phenotype correlation showed that the severity of clinical phenotype is related to the number of sister chromatid exchanges in the patients' chromosomes. Our findings revealed a dosage effect of the number of chromosomal affection on phenotype, thus confirming the reliability of karyotype as modifier of clinical phenotype, hence a marker for prognostic expectations and support both the additive and interactive hypotheses of karyotype/phenotype correlation.

P03.131 An Xp deletion detected prenatally by karyotype and defined by FISH and MLPA analysis

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Xp chromosome deletions in the female, predict the probability, but not the certainty, of an incomplete form of Turner syndrome and/or premature ovarian failure. The phenotypic effects may be extremely variable, from partial Turner Syndrome through minor menstrual abnormality, or even normal menses and fertility.

We used karyotype, FISH and MLPA analysis to investigate an Xp deletion, fortuitously diagnosed in a prenatal karyotype performed for advanced maternal age.

Initially, a terminal deletion was suspected from G-banded chromosome analysis, but FISH with a probe for Xp telomere region showed that the deletion was interstitial. MLPA with SALSA P018 and P106, confirmed the deletion and delineated the breakpoints at Xp22.13 and Xp11.4. Some remarkable findings with this technique were that the deleted region did not include STS (steroid sulfatase deficiency) and SHOX (short-stature gene), but it does include other important genes like IL1RAPL1 (responsible for X-linked mental retardation). Parents' karyotypes were normal, and patient's karyotype was redefined as 46,X,del(X)(p11.3p22.2)dn, indicating a de novo occurred interstitial Xp deletion with breakage and reunion at Xp11.3 and Xp22.2, of about 20Mb in size. The deletion, being ≥15Mb, would show completely skewed inactivation, and thus, no clinical manifestations of X-linked disorders.

Here we have described a case in which molecular cytogenetics (FISH and MLPA) can further delineate the size and position of the chromosome abnormality detected by karyotype. Those techniques supplement classical cytogenetics, obtaining a more precise and accurate information, which is critical for genotype-phenotype correlations and with important consequences for prenatal genetic counselling.

P03.132 46,XY karyotype with a duplication on the short arm of chromosome X in a girl with mental retardation and dysmorphic features.

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We present cytogenetic and molecular results in a 7 years old girl, referred for genetic investigations due to clinical dysmorphic features (up-slanting, short palpebral fissures, small eyelashes, broad nose, short philtrum, thick lips, micrognathia, hypoplasia of great labias and congenital cataract), epileptic seizures, tetraparesis and severe mental retardation. Abdominal computed tomography revealed a normal uterus, the absence of ovaries and left suprarenal calcification. Classical and molecular cytogenetic investigations, performed on peripheral blood lymphocytes, consisted in GTG-banded karyotype, FISH and array-CGH (44K, Agilent).

Notably, a male karyotype was found, with an atypical large Xp as confirmed also by FISH analysis with centromeric probes for X and Y chromosomes [SE X (DXZ1), SE Y class q arm - Kreatech, Poseidon] and a BAC probe for SRY gene (RP11-400O10). Moreover, array-CGH showed a 25.7 Mb duplication on the short arm of X chromosome, with proximal and distal breakpoints at Xp21.1 and Xp22.31, respectively.

In conclusion, we appreciate that the above-described cytogenetic rearrangements raise many questions concerning the origin and their pathogenetic role, which remain to be elucidated.

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P03.133 Case report: complex mosaicism in 45,X/49,XXXXY male

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Objective: We report a patient with rare gonosomal abnormality. The proband was newborn male born to a 27-years woman from a second pregnancy. The first pregnancy has stood on 4-5 week. The patient's father, who is 28 years old, is phenotypically normal 46,XY male. Perinatal hypoxic-ischemic brain injury was diagnosed in the patient. The proband was referred to genetic examination because of dysembryogenesis stigmas. Following phenotypic features: backward sloping foreheads, high nasal bridge, microretrognathia, were prominent. The patient had male genitalia descended in the scrotum testes and deformed penis. No pathology was revealed by abdominal ultrasonography and ophthalmologic examination.

Materials and Methods: Chromosome analysis was performed on cultured PHA-stimulated peripheral blood lymphocytes with GTG- and C-staining in accordance with standard techniques. FISH analysis was performed on interphase nuclei using standard protocols with CEP X/CEP Y DNA probes. Complete AZF deletions were tested according to Laboratory guidelines for molecular diagnosis of Y-chromosome microdeletions (EAA/EMQN, 1999), with some modifications. Partial AZFc deletions were detected by multiplex PCR of following STS loci: sY142, sY1197, sY1192, sY1291, sY1206, sY1054 and sY1125.

Results: Chromosome analysis shown 45,X[6]/49,XXXXY[19] karyotype in the patient. FISH analysis detected complex mosaicism with 5 cell clones: 45,X(21.2%)/46,XY(1.2%)/47,XYY(7%)/48,XXXX(14.1%)/49,XXXXY(56.5%). No complete (classic) Y-chromosome microdeletion was found. Partial AZFc deletion, b2/b3 was detected in both the patient and the patient's father. Revealed in the patient complex gonosomal mosaicism is the result of postzygotic errors of sex chromosome segregation, possibly because of instability of the structural rearranged Y chromosome.

P03.134 Fine mapping of FRA14B and FRA14C revealed new large common fragile site (cFS) genes frequently rearranged in different human cancers

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Common fragile sites (cFS) are evolutionary conserved chromosomal regions that are most prone to breakage under conditions partially

inhibiting DNA synthesis. These regions are found in all individuals and appear to be hotspots of chromosomal rearrangements in cancer. Approximately 90 cFS regions have been cytogenetically identified, but only few of these have been determined at DNA sequence level and completely characterized. Major part of examined cFSs coincides with extremely genomically large genes. The identification of the full repertoire of Human Fragilome sequences is and an important task of Cancer Genome research leading to the discovery of new cancer susceptibility genes.

Using six-color FISH-mapping with BAC-probes on metaphase chromosomes of aphidicolin-treated lymphocytes we have specified the locations of two aphidicolin-inducible cFS, *FRA14B* and *FRA14C*, and narrowed them down from chromosome bands 14q23 and 14q24.1 to smaller regions of 600 kb and 800 kb, respectively. Both cFSs span genomically large genes, gephyrin (*GPHN*, 670kb) and neurexin-3 (*NRXN3*, 1,6Mb), encoding neuronal proteins.

To assess the possible role of *FRA14B* and *FRA14C* in cancer chromosome rearrangements we performed fine-tiling oligonucleotide array CGH. We have detected multiple breakpoints within these two cFS occurring in neuroblastoma, melanoma, glioma, colon cancer and breast cancer cell lines and primary tumors. Subsequent validation of CGH results by six-colour FISH with BAC probes revealed additional balanced rearrangements at cFSs loci.

Our data enlarge the list of fine-mapped cFSs of the Human Fragilome and lead to the identification of two large cFS genes potentially associated with cancer.

P03.135 Molecular cytogenetic characterization of an interstitial 6q25.2q26 deletion in a 4-year-old girl with severe developmental delay, corpus callosum agenesis and hypertrichosis.

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Microdeletion of 6q25.2q25.3 was recently described in four patients with developmental delay, microcephaly, agenesis of the corpus callosum, hearing loss, and dysmorphic features (Nagamani et al., EJHG, 2009).

We describe a 4-year-old female patient who was shown by array-CGH (Agilent 244K) to have a heterozygous *de novo* 9.46 Mb deletion in 6q25.2q26.

The girl was born by caesarian section at 38 weeks of gestation after an uneventful pregnancy. Birth weight was 2870 g (p10-50), length 46 cm (p10-50) and head circumference 33 cm (p50). Examination at 3 months revealed severe hypotonia, umbilical hernia, large ears, and macrostomia. Major xerosis of hands and feet complicated by bouts of eczema as well as hypertrichosis (face, back, upper limbs) were also noted. Ophthalmological and audiological examinations were normal. Brain ultrasound and MRI at 4 months showed agenesis of the corpus callosum without other abnormality. At the age of 4 years, severe psychomotor delay (walking not yet achieved and absent speech) and microcephaly were noted, as well as mild growth retardation (weight and height slightly below 3rd percentile).

The region of overlap in 6q25.2q25.3 between the previously-reported patients and our case is 3.52 Mb long and harbours 12 protein-coding genes. Among these, *TIAM2*, *NOX3* and *SYNJ2* are candidates for normal development of the brain. Haploinsufficiency of any of these genes could be causative of mental retardation, microcephaly, and/or corpus callosum agenesis. In addition, a locus for hypertrichosis could be situated in the deleted interval.

P03.136 Molecular cytogenetic characterization of interstitial 5q14.3q15 deletions in two patients with severe developmental delay, hypotonia and seizures.

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Interstitial deletions of 5q are rare events. Only three independent studies reported on cases of 5q14.3q15 deletions in patients with severe mental retardation, muscular hypotonia, and epilepsy (Cardoso et

al., 2009; Engels et al., 2009; Le Meur et al., 2010).

We describe two unrelated patients with *de novo* overlapping micro-deletions in 5q14.3q15, spanning 5.73 Mb and 1.94 Mb, respectively. Patient 1 showed at the age of 4.5 years severe developmental delay, absence of speech, poor visual contact, stereotyped behaviour, ataxia, and epilepsy. Patient 2 was referred at the age of 10 months because of psychomotor delay and severe hypotonia. At the age of 2.5 years, he still did not walk or speak. EEG showed epileptic alterations from the age of 9 months and seizures occurred thereafter during 5 febrile episodes.

The boundaries of the deletions were different in the two patients, but an overlapping region of 1.84 Mb in 5q14.3 was defined. This region includes six genes: *CETN3*, *MBLAC2*, *POLR3G*, *LYSMD3*, *GPR98*, and *MEF2C*. Among those, three can be considered as particularly interesting in relation with the phenotypes of our patients: *GPR98* already reported to cause febrile and afebrile seizures (Nakayama et al., 2002), *MEF2C* recently shown to be responsible when haploinsufficient for severe mental retardation, poor eye contact and seizures (Le Meur et al., 2010), and *LYSMD3* which is highly expressed in the central nervous system.

J03.1 Hypersexuality, mental retardation and infertility in a 47,XYY patient

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The phenotype of 47,XYY males is normal, but the subjects are often tall. The majority of them are usually fertile, but genital hypoplasia, testicular ectopy and hypospadias have been also reported. They can have semen parameters ranging from severe oligozoospermia to normozoospermia. There is a definite correlation between this syndrome and committing offenses. It has been suggested that 47,XYY males have an increased risk of behavioral problems, poor social integration, low tolerance to frustration, emotional instability, aggressivity, and poor self-control.

A 26-year-old mentally retarded man referred to our department. His parents were non-consanguineous, and at birth, his father and his mother were 55 and 45, respectively. He was the last child and he has two normal brothers, five normal sisters. One of his sister and one of his nieces were also mentally retarded.

His clinical features were included tall stature, mild gynecomastia, small testes, as well as temper tantrums, aggressive and defiant activity. He has unusual elevated sexual desire caused a big problems such as sexual assaults on his mother and his sisters, masturbation addiction and masturbating in front of others and on the public areas. Therefore, he is kept in the restricted mental hospital.

His IQ was 65. Endocrinological testing demonstrated increase in the FSH and LH, and decrease in the testosterone levels. His seminal analysis revealed azoospermia. Conventional cytogenetic analysis using GTG-banding technique revealed the karyotype of 47,XYY in all his cells.

J03.2 Androgen insensitivity syndrome and inversion of chromosome 9: in one family

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Androgen insensitivity syndrome is a set of disorders of sex development caused by mutations of a gene encoding the androgen receptor. A person with complete AIS is a phenotypic female with a chromosomal genotype of 46,XY. This syndrome is an X-linked recessive and its gene is located at Xq11-12.

Pericentric inversion of the chromosome 9 is such a common minor chromosomal rearrangement which some cytogeneticists would consider them as normal variants. However, many reports in the literature raised conflicting views regarding the association with subfertility, recurrent abortion, and abnormal clinical conditions..

We are reporting a woman and two of her aunts which have the androgen insensitivity syndrome and inversion 9.

A 22-year-old woman was referred to our department due to primary amenorrhea and hirsutism. On physical examination, she measured 167 cm and weighed 61 kg. She was a female by appearance and external genitalia. Her axillary and pubic hair was scant (Tanner 2),

and she was rated Tanner 4 for her breast development. Her hormone assays showed normal FSH, mildly elevated LH and estradiol, highly elevated testosterone and DHT.

Her sonography showed absence of uterus and ovaries, and presence of aplastic testes in the abdomen.

Investigation of her family revealed that two of her aunts have also exactly the same clinical features.

Lymphocytes of the members of the family were cultured, and analyzed by use of the conventional G-banding technique. The karyotype of our proposita and two of her aunts revealed: 46,XY,inv(9)(p11q13). Her mother showed: 46,XX,inv(9)(p11q13) and her father was normal.

J03.3 Parity and Consanguinity of Down syndrome in Iran. A study of 402 free trisomic cases.

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Background: Down syndrome is the most frequent chromosomal abnormality in newborns with an incidence of around one per 700 live birth worldwide. Since the discovery of trisomy 21 in 1959 maternal and paternal age effect relation to the etiology and chromosomal pattern of affected persons has been studied. Since 1965 and with the establishment of the first Cytogenetics Lab. in the Country, we* have karyotyped over 700 Down patients. In this retrospective study parity and consanguinity of 402 free trisomic patients is analyzed and reported.

Materials and methods: Peripheral blood of 736 patients aged one day to 23 years were cultured and Karyotyped. Two hours colcemide treated cultures after 70 hours incubation and ten minutes KCl treatment were G-banded at 300-400 band level and analysed after air dry slide preparations. 35 well spread mitoses were analyzed by one of us* and seven mitoses were karyotyped. Results were reported to the in charge physicians according to ISCN nomenclature of 1995.

Results: Among 402 none mosaic free trisomic Down patients 53 percent were products of the first pregnancies, 29 percent were the results of the second pregnancies and the rest were outcomes of the 3rd to the 15th pregnancies. Only EIGHT per cent had consanguineous parents which is quite low as comparing to the average familial marriages in Iran which is over 15 per cent.

Conclusion: we believe that in Iran the risk of having a Down syndrome offspring in the first pregnancies is quite high and should be noticed by health officials.

J03.4 A report of a XYY male with hydrocephaly

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Here we are presenting the clinical finding in a five days old baby boy who referred to our Genetic Dept. due to undetectable testis and possible hydrocephaly. He was born premature at 32 weeks of gestation by cesarean. His parents were first cousin. He had a healthy sister. He had a feeding and respiratory problem. He passed away at age 10 day.

Chromosome study was performed using stimulated lymphocytes cells. Lymphocyte cultures from the patients were set up in RPMI 1640 supplemented with 20% FBS. high resolution chromosome banding was performed in all subject.

His karyotype revealed to be 47,XYY. From reviewing the article and case reports, we found that there least 4 other suspected cases of hydrocephaly whom with further clinical investigation appeared to be normal. We think that it is important to report our case to help the possible correlation between phenotype and 47, XYY karyotype.

J03.5 Report of a patient with isochromosome Xq and Robertsonian translocation 13;14

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Patients with isochromosome Xq phenotypically are resembled Turner syndrome with similar manifestations such as amenorrhea and infertility. Robersonian translocation between chromosomes 13 and 14 is

a common translocation among normal population with no obvious phenotypical effect. The combination of isochromosome Xq and the Robertsonian translocation 13;14 is a very rare event.

We are reporting a 30 years old single woman, who was referred to our department due to amenorrhea. Her clinical features were: short stature, broad chest, narrow palate, low posterior hairline, infantile nipples, undeveloped breasts, infantile external genitalia, absent of axillary hair, very scant pubic hair. Ultrasonography investigation revealed gonadal dysgenesis and hypoplastic uterus. Her IQ, cardiac and renal examinations were normal.

Cytogenetic analysis was performed by G-banding procedure, studying 30 metaphases of proliferating lymphocytes from her peripheral blood. Her karyotype was found to be: 45,X,i(X)(q10),der(13;14)(q10;q10).

We also studied her parents chromosomes to find out the origin of the translocation. It was revealed that her mother was normal, but her father carries the same translocation.

J03.6 Are there any association between 13q;14q Robertsonian translocation with Down's syndrome?

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There are contradictory data on the association of translocation (13q;14q) and Down's syndrome. Some researchers reported the association of translocations not involving chromosome 21 with Down's syndrome, and focused on the inherited 13/14 Robertsonian translocations and Down's syndrome. On the other hand, a study of four-generation kindred of 86 sibships was carried out when an index case had the Down's syndrome, due to trisomy-21, and also carried the 13q translocation. Their aim was to examine the risk of t(13q;13q) carriers having trisomy-21 children. They could not find any association.

We report a 21-year old healthy man who came for genetic counseling due to the Down's syndrome of his 23-year old sister. His father was 64, his mother 62, and both look healthy. His sister showed typical clinical features of Down's syndrome including: small stature, brachycephaly, flat occiput, flat facial profile, small ears, epicanthal folds, upslanting palpebral fissures, simian crease, hyperflexibility of joints, and mental deficiency.

Peripheral blood samples of the consultand, his sister and his parents were cultured, harvested and banded according to standard methods. Chromosome analysis of our consultand and his mother showed breakage and reunion at bands 13q10 and 14q10, and a derivative chromosome (13;14).

Consultand: 45,XY,der (13;14)(q10;q10)

Mother: 45,XX,der (13;14)(q10;q10)

Father showed normal karyotype.

Sister revealed trisomy-21 with 46 chromosomes due to the Robertsonian translocation between the chromosomes 13 and 14, as follows: 46,XX,der (13;14)(q10;q10),+21

The sister of our consultand was another rare case of Down's syndrome with trisomy-21 in association with inherited Robertsonian translocation(13q;14q).

J03.7 Short stature with t(11;22) and normal pituitary function

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Short stature and genetic disease is an interesting aspect in pediatric medicine. Several chromosomal diseases were reported with short stature. Previously t(11; 22) was reported with short stature and hypopituitarism. The present case was described is a case with short stature and translocation 11;22. She is a 14 years old normal appearing intelligent girl with no sign of puberty. In physical examination, her weight was 24Kg and height 127 cm, with normal blood pressure. There is no sign of cardiac or eyes abnormality. Laboratory findings were shown normal provocative growth hormone, estradiol in prepubertal range with normal thyroid hormone and cortisol level. Other routine tests were normal, but mild iron deficiency anemia (Hb10.7mg/dl) was seen. Father height was 155 and mother 163cm. Her birth weight was 3100gr and he mother complains growth problem of whom around first year of life. Two other children of family have normal growth. Her

cytogenetic study, on the basis of G-banding, showed 46XX, t(11; 22) (q23.3; q11.2). The t(11; 22) is the common non-Robertsonian constitutional translocation in humans. This translocation was reported by impaired anterior pituitary function and short stature, but this is the first case with similar translocation without gross pituitary dysfunction. A detailed clinical description of this patient, along with a precise cytogenetic designation of chromosomal breakpoint, allows further refinement of genotype-phenotype correlation for this translocation.

J03.8 A Down child with a de novo translocation

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A 2 year old girl was referred to our Genetic laboratory of being suspected to be Down Syndrome. She was born at term by natural delivery. Ultrasound performed during pregnancy but report the fetus to be normal. Her weight and head circumstances were within normal range. She had developmental delay. She did not gain weight from 6 months age.

Chromosome study was performed using stimulated lymphocytes cells. Lymphocyte cultures from the patients were set up in RPMI 1640 supplemented with 20% FBS. high resolution chromosome banding was performed in all subject.

Chromosome study was performed on stimulated lymphocytes of the case and parents. She found to be a Down child plus a de novo carrier of an apparently translocation between chromosome 3 and 20. Her karyotype was reported as 47,XX,+21,t(3;20)(p23;p11.2). There are few cases of a familial translocation plus a trisomy and a very few cases of de novo translocation and trisomy. Therefore, we think it is important to report our case.

J03.9 Translocation and trisomy 21 in a baby boy.

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A 7 months old baby boy was referred to our laboratory because of having clinical manifestation of Down syndrome. He was born at term by natural delivery. The eyes were oriental and attention was normal with normal reflection to sounds rolling as like as other babies without any other MR signs. His parent were unrelated. They had also such a delivery in family history.

Chromosome study was performed using stimulated lymphocytes cells. Lymphocyte cultures from the patients were set up in RPMI 1640 supplemented with 20% FBS. high resolution chromosome banding was performed in all subject.

The karyotype was ascertained as :47,XY,+21,tr(12;15)(p13.3;q26.1). His parents had normal karyotypes

P04 Reproductive genetics

P04.01 Investigation of the 4977 bp mitochondrial DNA deletion in asthenozoospermic men

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Objective: Sperm motility is one of the major determinants of male fertility. There is increasing evidence that mitochondrial DNA (mtDNA) mutations in sperm may lead to infertility. Due to the loss of several vital OXPHOS genes, mtDNA deletions mainly affect high-energy demanding post meiotic cells. Since spermatozoa movement also requires great amount of energy, defects in mitochondrial respiratory function is assumed to cause a decline in motility and, consequently, decrease of fertility.

Design: Detection of mitochondrial DNA deletion in human spermatozoa.

Materials and Methods: We collected 240 semen samples from 240 infertile men with asthenozoospermia (a<25% or a+ b<50% (WHO 1999). The samples with sperm agglutination, high viscosity and increased numbers of seminal leukocytes (leukospermia) were excluded. Blood samples obtained from 46 healthy individuals were used as control.

DNA was extracted from each semen sample. The mtDNA 4977 bp

deletion, so called 'common deletion' was recognized by nested PCR-amplified fragments in agarose gel electrophoresis.

Results: The 4977 bp deletion was identified in 96% of analyzed samples. Sperm motility (a+b) extremely varies (3%-38%) in semen samples with mtDNA mutation. The deletion was detected also in 87% control group blood samples. 13 bp direct repeats located at flanking region (8470-8482 and 13447-13459 respectively) may cause PCR slippage and artificial fragment amplification.

Discussion: The results of our study correspond to the current data confirmed that nested PCR doesn't allow detecting mtDNA deletion 4977 accurately. Potential use of this mtDNA deletion as a diagnostic test requires more reliable methods.

Support: none

P04.02 Molecular diagnosis of dominant and recessive spinocerebellar ataxias

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Autosomal dominant ataxias (SCA1-2-3-6-7) and autosomal recessive Friedreich's ataxia (FRDA) are the most frequent ataxias in Europe. Mutations in SCA1-2-3-6-7 are CAG expansions that are transcribed and translated into a long sequence of polyglutamine in the corresponding proteins, resulting in a toxic function for the cell. PCRs were performed with pairs of specific primers for each SCAs, in which the forward primer was usually fluorescinated. PCR products were analyzed by gel electrophoresis and by capillary electrophoresis using ABI PRISM 3130xl gel Analyzer. Liz500 was used as internal molecular weight standard and GeneScan Software was used to elaborate the data.

FRDA is a mitochondrial disease with a prevalence of 2×10^{-5} and a carrier frequency of 1/100. Almost 98% of the alleles has a pathological expansion of GAA in the first intron of the gene causing reduction of frataxin in homozygotes. Missense and truncating mutations are present in the remaining 2%. FRDA affected or carriers were identified by Hot-Start amplification using the XL-PCR kit (Applied Biosystems), with the following primers:

GAA-F(5'GGG ATT GGT TGC CAG TGC TTA AAA GTT AG3')
GAA-R(5'GAT CTA AGG ACC ATC ATG GCC ACA CTT CGG3').

PCR products were analyzed on agarose gel and by capillary electrophoresis ABI PRISM 3130xl gel Analyzer. To confirm the diagnosis, Rox1000 and Liz500 were used as molecular weight standards and GeneScan Software was used to elaborate the data.

40 patients were analysed of which two resulted SCA2 affected, one was SCA1 affected, two were Friedreich affected and seven were Friedreich carriers.

P04.03 Homozygous c.144delC of AURKC detected by bidirectional sequencing

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Molecular investigation by bidirectional sequencing of the exon 3 of AURKC gene using the couple of primers: TACCCTACCTCCCAAGCT-GA and CTTCAGGGCCACAATGAAAT, is performed for two Libyan brothers who complain from infertility with large-headed polyploid multi-flagellar spermatozoa. Clinical history showed absence of parental consanguinity and failure of many attempts of ICSI and IMSI. Genetic analysis showed the homozygous c.144delC for the two patients. This mutation leads to the production of large-headed polyploid multi-flagellar spermatozoa and causes meiosis I arrest in infertile men.

We underline that screening for c.144delC mutation of AURKC gene should be offered routinely to patients with macrocephalic sperm head syndrome in our countries (carrier frequency of 1/50), by setting up a rapid molecular test. Consideration of AMP treatment remains still not clear and will be discussed.

P04.04 Chromosome mosaicism in males with idiopathic azoospermia

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Introduction: Dicentrics are among the most common structural abnormalities of the human Y chromosome, and the phenotype is difficult

to predict, because it depends on different factors like duplications or deletions or the degree of mosaicism (45,X cell line). Clinical presentations in these patients can range from a classical female Turner phenotype, through mixed gonadal dysgenesis, to phenotypically normal males.

The essential genes for spermatogenesis are in Y chromosome and, because of that, karyotype is very important in males with idiopathic azoospermia (IA).

Case: A male with IA was referred to our laboratory for cytogenetic analysis.

Cytogenetic result showed the presence of a mosaicism 45,X[50]/46,XY[8]; chromosome Y was normal by GTG banding techniques but very small.

The result for the screening of STS markers showed a microdeletion in AZFb and AZFc intervals.

We also evaluated the percentage of mosaicism in another tissue; buccal smear's cells were selected. A normal male result in 100% of the cells was observed. Finally, SRY was studied by FISH in peripheral blood chromosomes and showed two red signals, which meant the patient had an isodicentric Y chromosome with common break point. Actually the karyotype was 45,X[50]/46,X,idic(Y)(q11.1).ishYq(SRY++)[8]. **Conclusions:** This case report underlines the necessity of a full-scale genetic analysis in all patients with reproductive failures. This case also aimed to establish the influence of sex chromosome mosaicism on the resulting phenotype respecting to differential tissue distribution, effects of the SRY and the breakpoints in the AZF region. Our results are in agreement to others reported.

P04.05

CFTR gene mutations and non-obstructive azoospermia: An Iranian study

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Genetic factors cause about 10% of male infertility. Cystic fibrosis conductance regulator (CFTR) gene mutations are among relatively frequent causes of male infertility. The aim of the present study was to evaluate the effect of CFTR gene mutations in non-obstructive azoospermia.

The incidence of common CFTR mutations including delF508, G542X, N1303K, W1282X and R117H was investigated in 106 Iranian patients with non-obstructive azoospermia using ARMS-PCR and PCR-RFLP methods. Also probable mutations in exons 4, 7, 10, 11, 20 and 21 of the CFTR gene were studied using SSCP, TTGE and sequencing methods. 50 fertile men were also studied as control group.

Thirteen patients (21.26 %) showed 406-6T>C, A120T, I148T, ΔF508, G542X, and IVS8-5T mutations. None of the mutations were observed in the control group. No significant statistical correlation was observed between the incidence of M470V polymorphism and male infertility ($P = 0.755$).

The present study indicates that there is a relation between CFTR gene mutations and developing non-obstructive azoospermia. Therefore, couples undergoing assisted reproductive technologies such as intra cytoplasmic sperm injection (ICSI) are advised to be screened for CFTR gene mutations.

P04.06 DETECTION OF CFTR MUTATIONS IN GAMETE DONORS IN SPAIN

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Mutation of CFTR protein can originate CF and CAVD; it is a recessive disorder and because of its high prevalence (1/25 in Caucasian population) it might be a good candidate for screening in gamete donors for assisted reproductive techniques. Our objective was to study the prevalence of CFTR gene's mutations in Spanish gamete donors from five reproductive centers.

Blood DNA samples from 588 healthy, both male and female donors,

ranging in age 18-32, were studied. The donors were from two reproductive centers in the North of Spain and three more from the East. DNA analysis was performed using a commercial kit from Innogenetics that covers, roughly, 80% of the mutations of European Caucasian population.

The incidence of CFTR mutations in donors was 3.9% higher in the North than in the East (4.32% vs. 3.21%). The premutation genotype 5T was present in 9.8% of the individuals (10.63 vs. 9.63, respectively). These differences were not statistically significant. The most frequent mutations were ΔF508 (42%), I148T (15.6%) and G542X (10.6%).

Our data show some differences with previous reports in the Spanish population, such as a relatively high prevalence of the I148T mutation, and an unexpectedly high prevalence of the 5T genotype. The 5T/TG variant decreases the efficiency of intron 8 splicing and its effects depend on other associated mutations, if any. Since a relatively high number (13.7%) of the individuals should not be accepted to be gamete donors, it is highly recommended to screen the candidates for CFTR gene mutations, including 5T analysis.

P04.07 AGORA, a biobank of congenital malformations and childhood cancers

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The aetiology of many congenital malformations and childhood cancers has not been established, but is most likely interplay between genetic and environmental risk factors. Large series of patients are needed to identify genes involved, and biobanks are a key resource for this purpose. Therefore, we have set up a biobank of congenital malformations and childhood cancers at the Radboud University Nijmegen Medical Centre (RUNMC) named AGORA, Aetiological research into Genetic and Occupational/environmental Risk factors for Anomalies in children. In almost every paediatric department of the RUNMC, a reference centre for 5 million people in the Eastern part of the Netherlands, patients and their parents are asked to donate blood or saliva for DNA isolation and to fill out questionnaires addressing the pregnancy period. Approximately 75-80% of the invited families participate and we already obtained 9150 DNA-samples (\pm 3350 case-parent-triads) and 2150 questionnaires. Currently, we are starting up the recruitment a random sample of Dutch children as a control group through the nationwide population administration. The availability of large cohorts of well-phenotyped patients within this biobank has enabled us to identify two new genes involved in the aetiology of hypospadias and renal anomalies, emphasizing the significant value of the AGORA biobank. Expansion of the biobank with Dutch, European and worldwide collaborators is set up for more rare disorders, such as specific anorectal and renal malformations. A significant challenge in this pursuit of genetic and environmental basis of congenital disorders is the efficient and coordinated collection of genotype, phenotype and environmental data.

P04.08 Cytogenetic study for couples with reproductive problems

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During last ten years, 278 married couples came to Center for genetics of Medical faculty-University of Sarajevo, for chromosomal cytogenetic analyze due to infertility or larger number of spontaneous abortions. To each married couple, karyotype has been made, using GTG technique from cultures of lymphocytes from peripheral blood.

Results: 25 (9%) of 278 had one partner carrying a chromosomal changes. Chromosomal changes at men were: 4 (1,43%) translocations, 4 (1,43%) marker chromosomes, 1 (0,35%) numerical chromosomal changes in mosaic types. Analyzes at women, have reviled larger number of chromosomal changes than at men. Changes found were: 4 (1,43%) translocations, 3 (1,08%) inversions, 2 (0, 71%) marker chromosomes and 7 (2, 51%) numerical chromosomal changes in mosaic types.

P04.09 Germline rearrangements in the tumour suppressor genes WWOX and FHIT are associated with Disorders of Sex Development

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Disorders of Sex Development (DSD), ranging in severity from genital abnormalities to complete sex reversal, occur in \sim 0.5% of live births and represent a major paediatric concern. In many cases it is not possible to make an accurate molecular diagnosis, with consequences for both treatment and genetic counseling.

We have used whole genome microarray copy number variation (CNV) analysis in an attempt to identify causative mutations in a cohort of DSD patients. In one 46,XY patient with ambiguous genitalia we detected a multi-exon deletion within the WWOX gene, which was also seen in the 46,XX mother. In another 46,XY patient with ambiguous genitalia we identified a single exon deletion within the FHIT gene.

Both WWOX and FHIT are known tumour suppressor genes, and to the best of our knowledge the deletions we have identified represent the first germline rearrangements affecting their coding regions. Both genes have been implicated as inhibitors of the Wnt/beta-catenin pathway (a critical component of ovarian development), and a previously described knock-out mouse of Wwox showed gonadal abnormalities in both sexes.

We are currently exploring the functional consequences of the identified mutations on the Wnt/beta-catenin signaling pathway, as well as determining the frequency of WWOX and FHIT mutations in a larger cohort of DSD patients.

P04.10** Endometrial gene expression analysis in infertile women in natural and artificial cycles

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We aimed to investigate endometrial gene expression profile in women with unexplained infertility in comparison with fertile controls at the time of embryo implantation in order to find potential predictive markers of uterine receptivity and to identify the molecular mechanisms of infertility. Further, we aimed to compare the gene expression pattern of receptive endometrium in natural cycles and in artificial cycles in these infertile women, in order to provide additional knowledge in the complex process of artificial endometrial preparation, which could be helpful for improving the outcome of assisted reproductive techniques in infertility treatment.

We used high-density oligonucleotide gene arrays, comprising 44 000 gene targets for endometrial gene expression profiling ($n=5$ in each group).

Hierarchical clustering and principal component analysis showed a clear distinction in endometrial gene expression between study groups. Ingenuity Pathways Analysis showed dysregulation of gene pathways involved in leukocyte extravasation signalling, lipid metabolism, and detoxification in the endometria of infertile women. In the artificial cycle, significantly high levels of cytokine activity and hormone activity, with cytokine-cytokine receptor interaction pathways were affected. Our study provides new information on genes and pathways that may have functional significance as regards to endometrial receptivity and subsequent embryo implantation.

P04.11 Search for epimutations of maternal expressed gene MEG3 (14q32) in early pregnancy losses

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Genomic imprinting is an epigenetic phenomenon, which is involved in regulation of embryonic development and placental function. In general, paternally expressed imprinted genes enhance fetal growth whereas maternally expressed suppress it. Previously we have reported a tissue-specific loss of methylation in paternal expressed loci KCNQ1OT1 (11p15) and PLAGL1 (6q24-25) in 9.5% and 10.3% spontaneous abortions, respectively. At the same time, normal epigenetic

status of the maternal expressed genes *H19*, *CDKN1C* and paternal expressed gene *SNRPN* was found. The aim of the present research was estimation of epigenetic status of other maternal expressed gene *MEG3* (14q32) in samples of first-trimester spontaneous abortions. The product of this gene is a regulatory RNA, which is involved in inhibition of cell proliferation. Methylation-specific PCR of *MEG3* promoter was performed using DNA from extraembryonic mesoderm and chorion cytotrophoblast of 87 first-trimester missed abortions with abnormalities of cell proliferation. Thirty induced abortions were studied as a control group. Normal differential methylation of *MEG3* parental alleles was observed in all studied embryos. Our data indicates that methylation status of *MEG3* imprinted locus on the chromosome 14 has no apparent effect for disruption of early human embryogenesis. This study was partially supported by Federal Special-Purpose Program (' P303) and RFBR (' 08-04-01344).

P04.12 The genetics of gonadal mosaicism

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Gonadal mosaicism (GM) for chromosome abnormalities in otherwise asymptomatic carrier is not a rare condition. However there are many problems related to GM which remain to be elucidated, including its prevalence, mechanisms and timing of formation, causing factors, and phenomenon of female predominance among GM carriers.

An approach to determine the population rate of GM for trisomies of chromosomes without severe imprinting effect was developed recently [Genet Test 11:342]. A female predominance of individuals with GM for free trisomy and for rearrangements involving pericentromeric breaks may be explained by female-specific chromosome loss and female-specific centromere instability in early embryo development [AJMG 136A:413]. Strong female predominance found among carriers of GM for derivative chromosomes but not in carriers of balanced reciprocal translocations suggests male-specific selection against unbalanced rearrangements [EJHG 13(S1):135]. Both direct (origin of trisomy 21 (T21)) and indirect (advanced grandmaternal age) evidence allow to assume that significant proportion T21 GM carriers had been conceived as trisomics. Mosaicism transmission reported in 12 of 80 families with T21 GM carrier(s) suggests a predisposition to trisomic rescue. Typical male preponderance in affected offspring with either maternally- or paternally transmitted T21 indicates that meiotic events are not responsible for the skewed sex ratio in Down syndrome. Among unaffected offspring of female T21 GM carriers, there was no bias from population sex ratio, while a strong female excess among unaffected offspring of male carriers of T21 GM uncovers meiotic non-homologous co-orientation of chromosomes 21 and X in spermatogenesis [Mol Cytogenet, in press].

P04.13 Clinical application of preimplantation genetic haplotyping for monogenic disorders in Czech Republic: Huntington disease, a case report.

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Preimplantation genetic haplotyping (PGH) for preimplantation genetic diagnosis (PGD) of monogenic disorders has been used at our centre since 2007.

Genetic haplotyping technique allowed to use the testing of multiple loci using standard DNA-based PCR protocols on products of multiple displacement amplification (MDA) from one blastomere biopsied from the cleavage-state embryo. Twenty seven gene-specific multiplex have been developed. Now they are ready to be used for the couples at risk of these monogenic disorders: cystic fibrosis, Huntington disease, Charcot-Marie-Tooth X-linked and type 1A, Duchenne/Becker muscular dystrophy, Haemophilia A, SCIDX1, Dystrophia myotonica 1, spinal muscular atrophy 1, polycystic kidney disease (ARPKD), connexin disorders associated with deafness, RHD typing (D antigen), BRCA1, HSAN1, Pelizaeus-Merzbacher disease, Neurofibromatosis type I, epidermolysis bullosa, propionic acidemia and Marfan, Lynch, Alagille, Curarino, Treacher Collins, IPEX, Crouzon and Fragile-X syndrome. The list of diagnosis where we can offer PGD/ PGH is be-

ing continually extended.

We demonstrate a case report of successful PGH for Huntington disease.

Genetic testing for an inherited predisposition to specific disease brings a deep dilemma for people with positive family history and determined reproductive period. PGH provides an efficient and successful alternative within prenatal diagnosis services and it counts among the methods reducing individual risk.

P04.14 The investigation of *AZF*, *CFTR*, *AR* genes in patients with impaired spermatogenesis

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Male infertility is found to have a role in approximately 50% of infertile couples. Microdeletions of the long arm of the human Y-chromosome are associated with spermatogenic failure. Mutations in the *CFTR* gene are also involved in male infertility. The androgen receptor mediates androgen action determining male sexual phenotypes and promotion of spermatogenesis.

Y-chromosome microdeletions, mutations and IVS8-nT sequence of *CFTR* gene and CAG-repeats number of exon 1 of the *AR* gene we have screened in 125 patients with azoospermia (n=53) and oligozoospermia (n=72) and in control groups by PCR-based methods. For each patient, PCR analyses were performed on DNA isolated from leukocytes derived from peripheral blood.

Microdeletions were detected in 10 of total 125 (8.0%) infertile men. Mutations in the *CFTR* gene were detected on 9 out of 125 analyzed infertile men. The most common mutation was F508del (7 of total 9 mutations). IVS8-5T allele of *CFTR* gene associated with congenital bilateral absence of the vas deferens was detected in 20 of total 125 (16.0%) of patients.

The frequency of alleles with CAG-repeats ≤ 17 was significant higher ($P < 0.05$) in group of patients with azoospermia (9.4%) comparing with control group (2.7%). The frequency of alleles with CAG-repeats ≥ 26 was significant differed ($P < 0.01$) between group of patients with oligozoospermia (19.4%) and control group (2.7%).

Molecular-genetic analysis of the *AZF*, *CFTR* and *AR* genes mutations as well as genetic counseling are necessary very important of diagnostics for patients with male infertility, especially if they are included in an assisted reproductive technologies program.

P04.15 Analysis of sperm chromosomal abnormalities and sperm DNA fragmentation in infertile men

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Objective: The aim of this study was to evaluate levels of sperm nuclear DNA fragmentation and of chromosomal abnormalities in a population of infertile men with severely teratozoospermia.

Materials and methods: The level of DNA fragmentation was determined by TUNEL assay in infertile men with severely teratozoospermia ($n = 70$) and in adult healthy fertile men (control group, $n = 30$). Three color fluorescence *in situ* hybridization was performed with probes specific for chromosomes 8, X, and Y in the control group and teratozoospermic men.

Results: Ours patients with severely teratozoospermia showed a significantly higher abnormality with total rate of 6.31% for chromosome X, Y and 18 in their spermatozoa compared to control group (1.52% ; $p < 0.0001$). A significantly higher proportion of total sperm DNA fragmentation was detected in patient with teratozoospermia (32.7% \pm 5.1%) compared to the control group (8.19% \pm 2.1% ; $p < 0.0001$). There were significant correlation between sperm DNA fragmentation and abnormal sperm morphology ($r = 0.75$; $p < 0.0001$). Aneuploidy rate was correlated to abnormal morphology, macrocephalic heads and abnormal flagella ($p < 0.001$). Moreover a positive correlation was found between the rate of sperm chromosomal aberration and the rate of sperm DNA fragmentation ($r = 0.45$; $p < 0.001$).

Conclusion: The result indicates that spermatozoa from patients with teratozoospermia contain greater DNA fragmentation and chromosomal aneuploidy and may lead to male infertility.

P04.16 Repeated genetic counselling in the family with infertility, Cystic fibrosis and chromosomal aberration - case report

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In pairs with reproductive failure we perform genetic counselling, cytogenetic examination using karyotyping of lymphocytes from peripheral blood and DNA analysis of most frequent mutations in CFTR gene in Czech population. In men with severe oligospermia or azoospermia we perform DNA analysis of AZF a, b, c regions on Yq. In men who carry balanced chromosomal translocations we determinate sperm aneuploidy and frequency of chromosomally unbalanced sperm using fluorescent in situ hybridisation. This examination helps to clarify the genetic prognosis and recommendations of appropriate methods of prenatal genetic diagnosis or preimplantation genetic diagnosis.

We describe repeated genetic counselling in the couple with reproductive failure. We confirm the CFTR genotype: p.[F508del]+[Q685PfsX4] in son from the pregnancy after IVF.

Within the preventive genetic testing of CFTR gene in this family we found chromosomal translocation in the mother's younger brother and we recommended examinations of sperm aneuploidy and cytogenetic analysis in other relatives.

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P04.17 A pericentric inversion of chromosome 5 in a infertile man

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Infertility affects an ~ 15 % of couples and male factor infertility is ~ 50% of cases.

A 34 years old men with oligozoospermia attending assisted reproduction program referred to our clinic for genetic testing and counseling.

Cytogenetic analysis was performed according to standard methods (GTG banding, with a minimum of 400 bands) on cultured cells obtained from the patient peripheral blood and the result showed an abnormal karyotype 46,XY,inv (5)(pter-q13).

Chromosomal study is very important to predict probability of transmission of affected gamete to offspring. Thus, in infertile males with abnormal karyotype, preimplantation genetic diagnosis (PGD) aid in increasing outcome of pregnancy.

P04.18 Frequency of cystic fibrosis transmembrane conductance regulator gene mutations in infertile men

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It is well established that 60-70% of patients with congenital bilateral aplasia of the vas deferens (CBVAD) have mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Increased CFTR mutation frequency was also reported in males with non-obstructive azoospermia and oligoasthenoteratozoospermia, emphasizing the importance of screening for CF mutations not only in men with CBVAD, but in all men with reduced sperm counts. The aim of this study was to investigate the frequency of CFTR mutations in infertile males from the Republic of Macedonia. A total of 169 infertile men (73 with azoospermia, 46 with severe oligozoospermia, 23 with mild oligozoospermia and 27 with normozoospermia but unexplained couple infertility), as well as 136 fertile controls were included in the study. Five common CFTR mutations (DF508, G542X, N1303K, 621+1G>T and R117H) and IVS 8 polyT alleles were analyzed by a multiplex PCR and single nucleotide extension reaction using a SNaPshot multiplex kit. Six DF508 and one G542X mutations were detected among infertile men, while two DF508 mutations were present among fertile controls. The frequency of CFTR heterozygosity was slightly higher in all groups of infertile males (5.5% in azoospermia, 2.2% in severe oligozoospermia,

4.3% in mild oligozoospermia and 3.7% in normozoospermia) in comparison to the fertile controls (1.5%). The frequency of IVS 8(5T) allele was 4.1% in infertile men and 3.3% in controls. Two infertile men with CFTR mutation (one with obstructive azoospermia and one with mild oligozoospermia) were also carriers of a IVS 8(5T) allele.

P04.19 FSHB promoter polymorphism within Progesterone Response element is associated with serum FSH level in men

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Follicle stimulating hormone (FSH) stimulates and regulates spermatogenesis in males. FSH consists of alfa- ja beta-subunit, alfa-subunit is common for all gonadotropins, whereas beta-subunit coded by the FSHB gene (#MIM 136530; 11p13) is hormone-specific.

We identified a potential regulatory SNP (rs10835638; G/T), located within an evolutionarily conserved Progesterone Response element (Grigorova et al., 2008). Genotyping of the rs10835638 in a cohort of young men (n=554; age 19.2 ± 1.7 years) revealed an association with serum FSH level in men (ANCOVA: p=0.001). Compared to the wild-type homozygotes (GG), the heterozygotes (GT) and the homozygotes (TT) for the minor allele had on average 15.7% and 40% lower levels of FSH in their bloodstream, respectively.

In a consequent study we genotyped the rs10835638 in the cohort of infertile male patients (n=1,029, age 31.7 ± 0.2 years). A significant excess of TT homozygotes as well as GT heterozygotes was detected among infertile men compared to the young male cohort (p<0.05). Consistent with the previous data for a cohort of young men, we detected an extremely strong association between the carrier status of rs10835638 variants and serum FSH levels (ANCOVA: p<0.001). The median serum FSH values in the heterozygotes (GT) and the homozygotes (TT) for the minor allele were 20.6% and 48.5% lower compared to wild-type homozygotes (GG). Moreover, among the patients diagnosed with idiopathic infertility, TT-carriers had significantly smaller combined testes volume (ANCOVA p<0.05).

Rs10835638 (FSHB -211 G/T) is the first identified polymorphism, which determines male serum FSH level and affects male reproductive potential.

P04.20 Gene expression of YBX2 and the interacting genes suggests a common pathogenic network involved in male infertility

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Infertility affects about one in six couples and approximately half of them have a male component. Despite the large amount of data collected in the last years about the molecular genetic basis of male infertility still remains a large group of men with unexplained infertility.

Ybx2 gene has been identified as a central component in the regulation of spermatogenesis in mice but the potential role of its human orthologue YBX2 in human infertility is not well known.

To explore the possible association of the YBX2 gene with idiopathic male infertility in humans we developed quantitative real-time PCR assays for the YBX2 and its previously reported interacting genes: PRM2, ODF1, TP1 and ACTL7B.

Testis biopsy, 50 mg specimen of testis tissue, was obtained from patients with obstructive azoospermia (5 normospermic controls) and infertile male (10 with microdeletions in the long arm of the Y chromosome and 10 with idiopathic infertility) with the histopathology ranged from Sertoli cell only (SCO) to severe oligozoospermia.

Real time RT-PCR assays (QuantiTect SYBR Green PCR kit) reveal large reductions in the mRNA levels of YBX2, PRM2, ODF1, TP1, ACTL7B in all patients with spermatogenic defects except patients with idiopathic oligozoospermia. Patients with idiopathic azoospermia had similar pattern of gene expression as those carrying microdeletions suggesting a possible similar mechanism leading to infertility. Further studies are necessary to explore the functional roles of this network of genes in germ cell development.

P04.21 Four SRY- Positive 46,XX Male Cases with and without Y Chromosome Microdeletions

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XX males resemble individuals with Klinefelter's syndrome (47,XXY) in their general masculine appearance with azoospermia. They are infertile and have small atrophic testes. Ninety percent of these patients have Y chromosomal material including the SRY (testis-determining factor) gene. Y sequences are usually located on the distal tip of the short arm of the paternal X chromosome. We report four 46, XX cases referred to our department for azoospermia and primer infertility.

Cytogenetic, molecular cytogenetic and multiplex PCR for Y chromosome microdeletion analysis were performed on peripheral blood samples. STR analysis for X chromosome was made in one case who had two derivative X chromosomes.

Chromosome analysis showed a 46, XX karyotype and translocation of SRY from chromosome Y to chromosome X was identified by fluorescence in situ hybridization (FISH) in all cases. One of the cases had two distinct derivative X chromosomes containing Y chromosome fragments and Y chromosome microdeletion was not detected. Other cases had Y chromosome microdeletions of the studied STR regions. We concluded that the cytogenetic analysis should be performed in infertile men with azoospermia and detection of SRY is important for the clinical diagnosis of XX males. Although, azoospermia in these cases is explained by the absence of long arm material including the candidate gene family for spermatogenesis, presence of different sizes of long arm of Y chromosome could explain clinical discrepancies.

P04.22 Association of mannose-binding lectin genetic variations with genital tract infections and tubal factor infertility

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Upper genital tract infections can inflict ineligible immune response and cause Fallopian tube damage and concomitant female infertility. However, the exact role of host genetic variation in the development of tubal factor infertility is still not fully established. We selected five genetic variations in mannose-binding lectin gene (MBL2) involved in immune response modulation and assessed their association with tubal factor infertility by comparing genotype frequencies among 164 women with tubal factor infertility and 400 control individuals. In our study MBL2 low-producing genotypes were related to increased incidence of pathogens associated with genital infections (OR=6.40 95% CI 1.99-20.34). In addition, hyper-producing MBL2 genotype HYA/HYA and low-producing MBL2 genotypes were associated with susceptibility to tubal factor infertility (OR=2.12 95% CI 1.19-3.79 and OR=2.34 95% CI 1.17-4.69, respectively), whereas high-producing MBL2 genotype HYA/LYA had a protective effect (OR=0.28, 95% CI 0.11-0.75). Our data suggest MBL2 polymorphisms play a role in receptiveness to pathogens causing genital infections and contribute to susceptibility to tubal factor infertility.

P04.23 Maternal smoking during pregnancy induces mitotic chromosome instability in fetal tissues

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Smoking is known to be related to DNA damage, chromosome/genome rearrangements, and aneuploidy and is associated with cancer and pregnancy complications. However, effects of maternal smoking during pregnancy on either somatic genome or mitotic chromosome instability in human embryos have not been ever evaluated. Here, using interphase multiprobe FISH and DNA chromosome-enumeration probes we have analyzed the rate of aneuploidy in spontaneous

abortions (SA) in the first trimester among smoking and non-smoking pregnant women. Chorionic villi of 600 consecutive cases of 5-15 weeks gestation from women aged from 16 to 47 years (mean age: 30 years) were processed for molecular cytogenetic analysis. Chromosome abnormalities were found in 303 out of 600 cases (50%). Chromosomal mosaicism due mitotic errors was detected in 157 from 600 cases (26.2%). Among 463 non-smoking females chromosomal abnormalities were detected in 237 cases (51.2%) including 122 mosaic cases (26.3%). Among 137 smoking females (22.8%) chromosomal abnormalities were detected in 65 cases (47.4%) including 35 mosaic cases (25.5%). This indicates that the incidence of regular and mosaic chromosomal abnormalities was similar among SA exposed and unexposed to smoking. However, the level of stochastic (spontaneous/background) aneuploidy or chromosome instability index (proportion of cells with aneuploidy involving other chromosomes than those of the main chromosome abnormality per chromosome) was significantly increased in SA exposed to smoking (1.4-2.1%) as compared to unexposed ones (0.3-1.2%). We conclude that smoking has negative impact on fetal physiology through increasing mitotic chromosome instability manifesting as spontaneous aneuploidy. Supported by Philip Morris USA Inc.

P04.24 Mitochondrial tRNA threonine and proline mutations in repeated pregnancy loss

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Introduction: Mitochondrial transfer RNAs (tRNA) genes are essential components of protein biosynthesis. These genes are hotspots for mutations. These mutations are associated with a wide spectrum of human disease. Many genetic factors are known in assessment of repeated pregnancy loss (RPL). Because of the role of mitochondria and its maternal inheritance, analysis of tRNA^{Thr} and tRNA^{Pro} in women with RPL are good candidate for more genetic evaluation.

Material and Methods: The nucleotide variations of threonine and proline were investigated in 96 women with idiopathic repeated pregnancy loss. The related mitochondrial area was amplified using a polymerase chain reaction (PCR). The PCR products were demonstrated by 2% agarose gel electrophoresis, and all the positive samples were purified and verified by an automated DNA sequencing method.

Results: The sequence analysis revealed 4 mutations in tRNA^{Thr}. There were A15907G (2.08%), A15924G (3.12%), G15928A (10.42%) as the most common mutations and G15930A (3.12%) as a novel mutation. The result of tRNA^{Pro} sequencing showed the T15972C mutation in 1 woman (0.4%) as a novel mutation too.

Discussion: These tRNAs mutations can alter their steady state level and affect the structure of tRNAs. It results in protein synthesis defects and, in turn, mitochondrial dysfunction. The mutations of these genes may be of help in the assessment of RPL. Further study of an expanded series of these tRNA mutants will be recommended to describe their etiologic role in idiopathic RPL.

P04.25 H19 methylation in Müllerian aplasia.

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Müllerian aplasia (MA) is a congenital abnormality of the female genital tract presenting as absence of functional uterus and upper two-thirds of vagina. Apart from three MA patients with signs of androgen excess and a mutation in *WNT4*, the cause is unknown.

Hypomethylation of the *H19* imprinted control region (ICR), on 11p15.5, has previously been suggested to be associated with genital and skeletal malformations. In addition, two Finnish patients with Silver-Russell growth restriction syndrome (SRS) and MA show extreme hypomethylation (<9%) of the *H19* region. Thus we hypothesized that non-syndromic MA could also be associated with aberrant methylation of *H19*.

We have performed restriction site-specific methylation analyses by quantitative Real-Time PCR of the *H19* ICR region in blood-derived DNA samples from 104 Finnish MA patients, 19 healthy relatives and 20 healthy female controls. No significant differences in mean methylation values were observed between patients (48%, SD 8.7), relatives (46%, SD 4.7), and controls (50%, SD 10.8).

The results indicate that hypomethylation of *H19* is not associated with the MA phenotype in our patients. We cannot exclude, however, the contribution of other epigenetic changes in the development of MA. Our ongoing genome-wide methylation studies in MA patients using Illumina's HumanMethylation27 Bead Array will spread further light on the general methylation status in patients with MA.

P04.26 Screening for NLRP7 mutations in Tunisian women with sporadic hydatidiform moles: report of two novel mutations

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Hydatidiform mole (HM) is an aberrant human pregnancy characterized by hydropic degeneration of placental villi without embryo; it is commonly non recurrent. Familial recurrent HM is an extremely rare autosomal recessive condition associated with homozygous mutations in human NLRP7 gene on maternal locus.

Our aim in the present study was to look if either this mutation exists in Tunisian women with sporadic HM.

Material and methods:

Molecular analyses were conducted on genomic DNA extracted from the peripheral white blood cells of 38 Tunisian patients with sporadic HM. All samples were screened by direct DNA sequencing of all exons of NLRP7 gene. A high resolution melting (HRM) analysis or sequencing was performed on testing controls.

Results:

Two novel missense mutations in a heterozygous state were found, c.544 G>A (p.Val182Met) in one patient and c.1480G>A (p.Asp494Thr) in two patients. These two variants of mutations were lacking in 66 unrelated controls. Two other mutations, a heterozygous state as, c.1532A>G (p.Lys511Arg) and c.2156 C>T (p.Ala719Val), already reported, were also documented in 2 patients.

Conclusion :

In our knowledge, this is the first reported study of NLRP7 mutation in patients with sporadic HM. As homozygous NLRP7 mutations are thought to be associated with a failure status as the occurrence of recurrent HM, or conception loss; heterozygous type could be a risk factor for non recurrent mole.

P04.27 FISH reanalysis of embryos suspected as aneuploid by PGD for monogenic disorders

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Introduction: In PGD for monogenic disorders, 5%-10% of the embryos are suspected of being chromosomally imbalanced. That may be the result of true aneuploidy or represent artifacts of single cell PCR.

Aim: To estimate the rate of true aneuploidy by FISH reanalysis of PGD embryos, suspected of having chromosomal abnormalities, as assessed by single cell PCR.

Methods: PGD for monogenic disorders is performed by multiplex nested PCR for the causative mutation(s) and haplotype analysis. We have reanalyzed 35 PGD embryos suspected of being aneuploid, by spreading the entire embryo on day 5 and FISH hybridizing with 2-5 probes. Aneuploidy and mosaicism rates were evaluated.

Results: FISH results confirmed chromosomal aberrations in 21/35 embryos (60%). Of these, 2 were trisomic, 7 monosomic, and 12 presented a mosaic aneuploid pattern. The remaining 14 embryos (40%) were chromosomally balanced. 5/14 were considered as true monosomy in a single cell (i.e. mosaicism). 2/14 embryos, analyzed for an X-linked condition, were suspected of being 45, X, but FISH reanaly-

sis hinted for PCR amplification failure. In 1/14 embryos, results were compatible with allele drop out (ADO). In 6/14 embryos the most probable explanation was maternal cell contamination.

Conclusion: FISH reanalysis confirmed the PCR results in the majority of embryos suspected as aneuploid. We therefore suggest not transferring such embryos. Conflicting PCR results may be explained by high rate mosaicism, resulting from post-meiotic errors. However, incompatibility could also be attributed to contamination, amplification failure or ADO which may be reduced by the use of additional polymorphic markers.

P04.28 Preimplantation genetic diagnosis - 4 years' experience at the University Medical Centre Ljubljana

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Background: Preimplantation genetic diagnosis (PGD) offers early investigation of embryos in couples with a high risk for offspring affected by a genetic disease. We report indications and results associated with the PGD program conducted at Gynecology clinic Ljubljana from June 2004 to December 2008.

Methods: Retrospective analysis. Sixty cycles were performed in 34 couples enrolled in the PGD program. Embryos were biopsied on the third day and the genetic analysis was performed using FISH and PCR method. Embryo transfers were carried out on the fifth day.

Results: The main indication was chromosomal abnormalities (67%), followed by recurrent miscarriages (16%), autosomal dominant and recessive diseases (9%), and X-linked diseases (6%). Sixty cycles were performed and 48 embryo transfer procedures. There were 17 biochemical and 15 clinical pregnancies resulting in clinical pregnancy rate 25% per cycle and 31.25% per embryo transfer. A total of eleven unaffected children were born.

Conclusions: PGD is technically a very challenging procedure. Superior knowledge and communication between geneticists and reproductive medicine scientists is mandatory for successful PGD procedures. PGD has gained a place among the choices offered at Gynecology clinic Ljubljana to couples at risk of transmission of genetic disease.

P04.29 The paternal effect of chromosome translocation carrier in the embryos meiotic segregation.

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Introduction: Understanding the meiotic segregation mechanism of translocation is important is a crucial step for an estimation of the risk of either pregnancy loss or birth defects. The objective of this work is to estimate the meiotic segregation rates in preimplantation embryos from PGD cycles of female and male reciprocal translocation carriers.

Material And Methods: Fourteen couples with reciprocal translocations carried out 20 PGD-FISH cycles from January 2006 through July 2009. PGD was performed on 118 day 3 embryos using FISH with specific probes for each translocation. The meiotic segregation modes were estimated and the effect of the paternal origin of translocated carrier.

Results: Overall, 2:2 segregation was observed in 78 embryos (66.1%), 3:1 in 28 (23.7%), and 4:0 and chaotic patterns in 12 (10.2%). The segregation most frequently was alternate. According to the gender of translocation carriers, the frequencies of adjacent-1 and adjacent-2 were significantly lower in embryos from female than male PGD-cycles. For male translocations, alternate was the most frequent mode. The 3:1 was the most frequent in female translocations carriers.

Conclusions: We describe the segregation modes in male and female translocation carriers by FISH on preimplantation embryos. The production of unbalanced gametes was very different between the sexes. These data may be explained by differences in the frequency and localization of crossing-over. Analysis of the meiotic behaviour of chromosomes and the differences between the meiotic products of female and male for a chromosomal rearrangement could provide clues as to the respective roles of the mechanism in meiosis.

P04.30 The genetics of Premature Ovarian Failure**

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Premature Ovarian Failure (POF) is a complex disorder characterized by menopause before the age of 40 years. Its occurrence is about 1 % in the Caucasian population. A genetic basis for the disease has been demonstrated by the prevalence of familial cases but to date only a few of the genetic determinants for POF were identified. The aim of our study is to combine several genetic approaches in order to discover different genetic risk factors for POF.

Using POF case/control cohorts and a candidate gene approach we confirmed the role of the DIAPH2 X-linked gene, and excluded the INHA gene as a determinant of POF. We also showed that the DIAPH2 gene is one of the main determinants of cytoskeleton rearrangements associated with FSH stimulation of granulosa cells suggesting that alteration in cytoskeleton may be responsible for POF. Deep-sequencing study of POF patients is ongoing to identify DIAPH2 variants implicated in the disease.

From the study of the isolated populations in Italy, Val Barbera and Friuli-Venezia-Giulia in Northern Italy, and Carlantino in Southern Italy we identified 52 POF patients among 887 women in menopause. Whole-genome genotyping and association analysis showed promising preliminary results. Replicas in the Italian case/control cohorts are currently ongoing in order to further validate these findings.

P04.31 Polymorphisms in the thrombophilia genes are associated with severe preeclampsia (EPH - gestosis).

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Recently, it has been proposed that abnormalities in coagulation and fibrinolysis contribute to the development of preeclampsia by increasing the thrombotic tendency. This hypothesis was tested in women who have had preeclampsia (EPH - gestosis) compared with matched controls. Polymorphisms in the thrombophilia genes (PAI-1 4G/5G promoter polymorphism, MTHFR C677T polymorphism, FV G1691A polymorphism, FII G20210A polymorphism, eNOS VNTR polymorphism in 4 intron, TPA Alu repeat I/D polymorphism in 8 intron) were determined in women with severe preeclampsia (N=198) and women with uncomplicated pregnancy (N=206) in the populations from Bashkortostan (Russia). Allelic and genotypic frequencies of FII polymorphism ($p>0,05$) and TPA polymorphism ($p>0,05$) did not differ between both groups of pregnant women. The PAI-1*4G allele (OR=1,35), eNOS*4a/*4b genotype (OR=1,82), FV*G/A genotype (OR=2,93) and FV*A allele (OR=3,22), MTHFR*T/T genotype (OR=6,06) and MTHFR*T allele (OR=2,40) were associated with increased risk of severe preeclampsia. Based on these results, we recommend the incorporation of these four analyses in individuals with severe preeclampsia. These patients may benefit from the application of low molecular weight heparin as early as possible in the pregnancy in order to prevent uteroplacental microthromboses.

P04.32 Possible relation between 22s+ heteromorphism and recurrent pregnancy loss

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Polymorphic variations of heterochromatin (heteromorphisms) on chromosomes 13-15, 21-22 are described as heritable chromosomal variations without a proven impact on phenotype. Relationship between chromosome heteromorphisms and infertility is yet undefined but there are many reports of a high frequency of these variations among infertile men and couples with recurrent pregnancy losses.

The aim of this study was to report the 22s+ heterochromatic variants among cytogenetic abnormalities recorded in couples with recurrent pregnancy loss and to discuss the possible mechanism of how 22s+ might affect spermatogenesis through a documented observation.

Cytogenetic analysis was undertaken in 334 couples who were explored because 2 or more pregnancy losses. Only two male patients

(1,2 %) were found to have 22s+ heteromorphism. Familial investigation made for one case showed a maternal transmission of the variant. Sperm fluorescence in situ hybridization (FISH) was applied in the second men, who had two children, secondary infertility related to astheno-teratozoospermia and 3 early pregnancy losses after ICSI treatment. FISH analysis revealed an increased rate of sperm aneuploidy which can explain negative impact on ICSI outcome.

At genetic counselling, the couple has been informed about the place of preimplantation genetic screening to improve ICSI success.

We conclude that heterochromatin polymorphism in infertile males and couples with recurrent pregnancy loss seems to be more than an incidental finding, and must not be considered as a normal variant. Polymorphic heterochromatin may have deleterious effects on the genetic constitution of spermatozoa but more studies are needed to make clear an eventual relation ship.

P04.33 Functional evidence implicating FOXL2 in non syndromic premature ovarian failure and in the regulation of the transcription factor OSR2

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FOXL2 encodes a forkhead transcription factor whose mutations are responsible for the blepharophimosis-ptosis-epicanthus inversus syndrome (BPES), involving craniofacial/palpebral abnormalities often associated with premature ovarian failure (POF).

We describe a FOXL2 variant (p.Gly187Asp) in a case of POF without BPES. The subcellular localisation of FOXL2-G187D was normal but its transactivation capacity tested on two reporter promoters, one of which should be relevant to the ovary, was significantly lower than that of normal FOXL2. However, FOXL2-G187D was able to activate strongly a reporter construct driven by the promoter of Osr2 (odd-skipped related 2 transcription factor), which we have suggested to be a crucial target of FOXL2 in the craniofacial region. This is compatible with the absence of BPES in our patient.

Our data provide evidence in favour of the implication of FOXL2 variants in non-syndromic POF and confirm the regulatory interaction between FOXL2 and OSR2 whose perturbation might contribute to the palpebral abnormalities observed in BPES patients.

P04.34 Role of mitochondrial mutations in premature ovarian failure

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Premature Ovarian failure (POF) is defined as the cessation of ovarian function under the age of 40 years and is characterized by amenorrhea, hypoestrogenism and elevated serum gonadotrophin concentration (FSH). It is a heterogeneous disorder with a multicausal pathogenesis such as chromosomal, genetic, enzymatic, iatrogenic or infectious aberrations. But in idiopathic POF, involvement of unknown mechanisms may affect the rate of oocyte apoptosis. Studies have shown that oxidative stress (reactive oxygen species) affects the quality of gametes. Mitochondrial mutations in different complexes of electron transport chain have been reported to disrupt the electron flow which lead to formation of more superoxide ions or increased level of ROS. This study was aimed to analyze the mitochondrial genome of idiopathic POF patients. 30 POF diagnosed patients were enrolled in this study. Blood samples were collected from the patients and controls. DNA was extracted using phenol chloroform method. 24% variations were found to be non-synonymous and 76% were synonymous. It was found that 48% variations were in complex I, 8% in complex III, 24% in complex IV and 20% were in complex V of electron transport chain. We found most of the non-synonymous mitochondrial variations in complex I (48%) of the respiratory which is the largest of enzyme complex. The data suggest that the mitochondrial mutations (high ROS stress) may be the cause of follicular atresia which may lead to premature ovarian failure. The role of OS in female fertility and sub-fertility is an area deserving of continued research.

P04.35 ATPase6 mutation and oxidative stress is associated with premature ovarian failure (POF)

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Purpose: Premature ovarian failure (POF) is a heterogeneous, multi-factorial disorder. ATPase6 synthase helps to maintain the mt genome integrity and mutations in ATPase 6 are associated with overproduction of ROS in variety of diseases. **Methods:** This pilot study included 20 POF patients and 20 age matched healthy female controls. ROS levels in blood plasma were measured by chemiluminescence assay. mtDNA ATPase6 gene was amplified and sequenced from the blood lymphocyte DNA.

Results: 55% (11/20) patients showed nucleotide changes in ATPase6, as compared to 10% (2/20) in controls and all of these mutations were either G>A or C>T (mostly non-synonymous) except one case showing silent polymorphism A>G at nucleotide position 8679. Overall median RLU/min was found to be significantly (p=0.0037) higher in POF patients 50480 (120, 132966) as compared to controls 340 (120, 5094). Nucleotide changes were found to be positively correlated with ROS levels in the POF patients. Moreover, 50 % of the POF patients had high ROS levels, 20 % had medium and 30 % had normal values as compared to controls.

Conclusions: OS associated with ATPase6 gene instability may be the underlying aetiology in idiopathic POF. Screening other mt genes with large number of samples may help in better understanding the role of OS in POF. Thus the results from this preliminary pilot study highlight that mt gene (ATPase6) nucleotide alteration and supraphysiological ROS levels may play a causal role in accelerated atresia of germ cells and lead to premature ovarian failure.

P04.36 Dissecting the role of FMR1 mRNA in the molecular FX-POI pathogenesis

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CGG repeats expansion (55-200 units, permutation range) in the 5'UTR of FMR1 gene (Xq27.3) is associated with Fragile-X Tremor/Ataxia Syndrome and Primary Ovarian Insufficiency (POI). FMR1 premutation represents the most significant single gene variant associated with POI, however no studies are available to evaluate its pathogenic effect in the ovary context.

As for FXTAS, an RNA-mediated toxic gain of function could be hypothesized for FX-POI. By R-EMSA and RNA-pull down assays, 76ribo(r)CGG probe showed a higher binding affinity for several proteins derived from human granulose ovary (named rCGG-Repeat Binding Proteins, rCGG-RBPs) compared to the 28rCGG one. rCGG-RBPs were identified by means of MALDI-TOF analysis (Tab. 1). The interaction of endogenous FMR1 mRNA for identified rCGG-RBPs was tested in vivo by RNA immunoprecipitation; FMR1 premutated mRNA solely co-immunoprecipitate with HSP27 and CRYAB heat shock proteins, suggesting that in vivo premutated FMR1 mRNA differs from the wild type in making RNA-protein complexes. The role of HSP27 and CRYAB in the FX-POI pathogenesis was further investigated in an ovary cell model.

An "heat shock like" response in human ovary granulosa cells expressing expanded CGG mRNA was observed, strongly suggesting the toxic role of premutated mRNA itself in the ovaries.

This work is supported by Telethon grant GGP009126

Tab.1 List of ovary rCGG-RBPs identified by MALDI-TOF

| Protein | N° peptides |
|--|-------------|
| Splicing factor proline/glutamine rich gi4826998 | 5 |
| hnRNP A2/B1 gi4504447 | 11 |
| hnRNP F gi4826760 | 7 |
| hnRNP H gi1710632 | 14 |
| hnRNP M gi14141152 | 14 |
| hnRNP G gi542850 | 2 |
| hnRNP A1 gi133254 | 9 |
| hnRNP H3/2H9 gi23503095 | 17 |
| Unknown belonging to α -crystallin-type heat shock proteins gi2852648 | 3 |
| HSP27 gi11036357 | 4 |
| Chaperonin HSP60 gi31542947 | 3 |
| Nuclear cap binding protein gi158513766 | 4 |
| H1 histone family gi28839618 | 6 |
| Histone H2A.2 gi31979 | 7 |

P04.37 Use of Y chromosome specific repeat sequencing for sexing by single cell PCR and real time PCR

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Embryo sexing is one of the important ways for sex selection of offspring. This is a potential method to considerably improve animal breeding and the efficiency of dairy and meat production. A novel repeated sequence specific to male cattle has been identified and named S4 . S4 is a 1/5 Kb repeating unit contains various internal repeated sequence. S4 is localized on long arm of the Y chromosome in the region near to ZFY genes. Aim: The objective of this study was to establish a simple, sensitive, reliable, reproducible and cost effective PCR based technique for sexing.. Materials and Methods: Genomic DNA was extracted from the whole blood samples of 4 male and female cattles. PCR and real time PCR were performed using specific primers for this region. Result: By this PCR based methods we could differentiate between female and male genomic DNA. Real time PCR employed in order to have a quantitative method. Discussion:With this technique we can distinct male from female using as much as 1pg DNA. Using this method we could determine the sex of an embryo (4 blastomeres) . The Real Time PCR method optimized here was able to be used for the quantitative detection of Y chromosomes in semen.

P04.38 Genetic aspects of recurrent miscarriage

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Recurrent Pregnancy loss is the miscarriage of two or three consecutive pregnancies in the first of early second trimester. Although approximately 10-15% of all recognized pregnancies result in miscarriage, less than 5% of women will experience two consecutive miscarriage, and only 1% experience three or more. In recurrent pregnancy loss, Current Practice often fails to make a diagnosis, as the fetal causes of pregnancy loss are usually ignored and only the maternal factors are assessed. The maternal causes are well known and include: Uterine factors, Infections, Autoimmune syndromes, endocrine abnormality, all immune factors and possibly hereditary thrombophilia. The fetal causes of embryo loss include structural malformations that are incompatible with life, and Chromosomal aberrations. Unfortunately, no explanation is found in 50% to 70% of couples with recurrent pregnancy loss. About 5% of couples with recurrent pregnancy losses have a chromosomal abnormality and translocation is the most common inherited chromosomal abnormality. Although a parent who carries a translocation is frequently normal, their embryo may receive too much or too little genetic material. Couples with translocation or other specific chromosome defects may benefit from pre-implantation genetic diagnosis. But in fact 60% or more of early miscarriages may be caused by a random chromosomal abnormality, usually a missing or duplicated chromosome. These malformations are usually associated with a normal karyotype. Single gene disorders associated with recurrent miscarriage are myotonic dystrophy. The factor V leiden mutation is the most common genetic predisposition to thrombosis but its carrier frequency in the white population is 3-4%.

P04.39 Genetic Markers Related To Ovarian Response In Bulgarian Women With Reproductive Problems

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Background: recent studies showed a relation of the genetic polymorphisms in ESR1 and ESR2 genes together with FSH receptor variants with ovarian response during in vitro fertilization. However a population and ethnic specificity has been described. The previous studies showed a relation of genetics variants in the studied genes with polycystic ovarian syndrome in the Bulgarian population.

Aim: to investigate the ESR1 Pvu II and Xba II polymorphisms, ESR2 Rsa I polymorphism and Thr307Ala and Asn680Ser polymorphisms in Bulgarian women with reproductive problems.

Materials and methods: 72 women with reproductive problems and 58 healthy controls were investigated.

Results: The women with reproductive problems were categorized in three groups: with poor, boundary and normal ovarian response. We found unexpected low heterozygosity for ESR2 Rsa I polymorphism both in the group of patients and controls. For ESR1 XbaI polymorphism A/A (-/-) variant demonstrated association with poor ovarian response in the groups investigated ($p = 0.040$). Association has been found for the Thr307Ala polymorphism in the FSH receptor where the A/A (+/+) variant is significantly most common in the groups with better ovarian response ($p = 0.037$).

Conclusions: Larger studies are needed to establish the role of the ERS1 and FSHR polymorphisms for the ovarian response during assisted reproduction before they can be used for establishing of an individual protocol during stimulation.

P04.40 sperm deoxyribonucleic acid fragmentation is increased in failed fertilization and associated with a high risk of pregnancy loss after invitro fertilization and intracytoplasmic sperm injection.

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Introduction: Numerous recent studies involve DNA damages associated with poor fertilization rates, early embryo development defect, and poor quality of conceptus following assisted reproductive technologies (ART).

The aim of this study is to correlate any detected DNA damage with fertilization rates and with spontaneous abortion rates after IVF and ICSI.

Material and methods: Semen samples ($n = 25$) were collected from men who had a repeated unsuccessful ART cycle or recurrent miscarriage. For each sample a Pure Sperm density gradient centrifugation was performed and the percentage of recovered sperm with DNA fragmentation was determined with the use of terminal desoxyribonucleotidyl transferase-mediated deoxyuridine triphosphate biotin nick-end labelling (TUNEL) assay.

Results: Our patients showed a significantly higher percentage of sperm DNA fragmentation (21.04 ± 9.87) which can explain in the part the failed of ART cycle.

The sperm DNA fragmentation is increased in failed implantation group 23.9% versus 20.75% in the group with failure of fecundation after IVF and ICSI.

The DNA fragmentation index was significantly higher in the failed implantation group after in vitro fertilization 30% versus 17.83% after intracytoplasmic sperm injection.

The patient who showed recurrent miscarriage approved a high level of DNA fragmentation 19.45 %.

Conclusion: Sperm DNA damage is associated with decreased IVF outcomes; it may contribute to failure of pronuclear formation and embryo development. It is also associated with a significant increased risk of pregnancy loss after IVF and ICSI.

P04.41 +1040 C/T polymorphism in coding region of thrombin-activatable fibrinolysis inhibitor gene contributes to the risk of recurrent fetal loss

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Recurrent fetal loss is common health problem affecting up to 5% of women in reproductive age. It has been shown that plasma thrombin-activatable fibrinolysis inhibitor (TAFI) concentrations increase during pregnancy and return to baseline levels soon after delivery. The +1040C/T single nucleotide polymorphism in coding region of TAFI gene is associated with TAFI blood levels.

Our objective was to determine whether there is an association between +1040C/T polymorphism in TAFI gene and recurrent pregnancy loss.

Study was carried out in a group of 95 women (45 controls and 50 women with recurrent fetal loss). The +1040C/T polymorphism was detected by polymerase chain reaction, followed by digestion with specific restriction enzyme.

Increased frequency of +1040T/T genotype was observed in a study group, but without statistically significant difference. Carriers of T/T genotype have increased risk of fetal loss by 4-fold, compared to carriers of C/C (95%CI 0.768-20.819; $P=0.1$) and 4.22-fold compared to carriers of C/T genotype (95%CI 0.788-22.616; $P=0.09$). C allele is associated with reduced risk of recurrent pregnancy loss compared to T allele, (OR 0.67; 95%CI 0.355-1.249; $P=0.2$).

In conclusion, we observed increased frequency of +1040T/T genotype in a patient group, suggesting that this genotype could be potential risk factor for recurrent fetal loss. Further investigation should be carried out in order to establish definite role of this polymorphism in etiology of recurrent miscarriages.

P04.42 Substantive sex difference in fetal germinal Trisomy 21 mosaicism

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We have previously proposed that the underlying cause for the well-known parental sex difference in risk of having a child with Down syndrome is different degrees of fetal gonadal trisomy 21 (T21) mosaicism. To test this hypothesis we have performed fluorescence in situ hybridisation (FISH) using two chromosome 21-specific probes on fetal ovaries and testes, obtained from termination of pregnancy for a non-medical/social reason.

In the initial study we documented an average of 0.54% ovarian T21 mosaicism (range 0.20-0.88%; SD 0.23) in fetal ovaries from eight cases (Hultén et al. *Molecular Cytogenetics* 2008, 1:21). We have now followed up this observation by recording copy number of chromosome 21 in testes from four male fetuses. Analysing at least 2000 cells per case in a total population of nearly 12.000 we could not detect a single T21 cell nucleus (Hultén et al. *Molecular Cytogenetics* 2010, 3:4). This sex difference is highly statistically significant ($p<0.001$).

We conclude that the aberrant recombination patterns seen by family linkage most likely is related to meiotic pairing and recombination abnormalities in T21 oocytes. Thus, the risk in having a child with T21 DS is likely to be dependent on degree of gonadal T21 mosaicism, with the maternal age effect due to an accumulation of any such T21 oocytes during development from fetal life until menopause (Hultén et al. *Reproduction* 2010, 139:1).

P04.43 A new accuracy method to undocumented embryo diagnosis

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Introduction: The FISH technology has been applied to evaluate the chromosomal pattern of unipronuclear and undocumented embryos. However, this technique presents limitations; and we can not differ between paternal and maternal contributions.

The aim of this study was to ascertain the real ploidy of these embryos with an accuracy method, using DNA technology in order to increase the success rate per IVF-cycle or to prognose a next treatment in case of no pregnancy.

Materials And Methods: Since March 2009, 23 undocumented embryos were collected in our *in vitro* fertilization Unit. DNA from parents were obtained and isolated from bucal swab. Embryos were placed in alcaline lysis buffer. After neutralization, cells lysates were used directly for multiple displacement amplification (MDA).

In order to identify the paternal-maternal origin of the DNA from the undocumented embryos, the AmpF/STR[®] Identifiler[®] PCR Amplification Kit was used.

Results: The 23 undocumented embryos obtained had DNA amplification and positive paternity test. Eighteen embryos (78.27%) were disomic embryos (2n) with paternal and maternal contributions, and 4 of them (17.39 %) were 2n embryos with only maternal contribution. Only one embryo (4.34%) was haploid (n) or uniparental disomic with maternal origin.

Conclusion: We are able to discriminate between disomic embryos and partenogenic embryos with a common paternity test. Moreover, we could apply a comercial genotyping kit after MDA amplification.

Thanks to this, a great number of couples could benefit from these findings. To our knowledge, this is the first report evaluating undocumented embryos by means of a paternity test.

P04.44 Y;21 translocation in infertile male patient with 45,X karyotype

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30-year-old male patient has referred to our clinic for unravelling the underlying aetiology of the azoospermia. He had no unusual medical history. At physical examination, obesity, short neck and gynecomastia were noted. All studied hormon levels were normal but estradiol was 2-fold higher than upper limit. Having azoospermia in the spermiogram scrotal USG was in normal range. Cytogenetical analysis and FISH studies were performed subsequently and 45,X,add(21)(p10) and 45,X,add(21)(p10).ish der(Y;21) (q12;p10) were found, respectively. On C-banding, dicentric staining of translocated chromosome was observed. Molecular genetics studies using multiplex PCR revealed the presence of Y chromosome sequences at SRY, AZFa, AZFb, AZFc regions.

J04.1 The social homogamy in Morocco

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The study of the social homogamy, which is the tendency to marry from the same social group, derives its importance from the fact that the choice of the spouse has consequences not only on the couple, but also on the descendants. Indeed, the choice of the spouse influences the genetic structure of the family and guides the evolution of the hereditary patrimony of the population.

To assess the level of social homogamy in the region of Souss-Massa-Darâa in southern Morocco and to determine to what extent this rule of marriage marks the population of this region, a prospective study was carried out through a survey of 194 families sampled randomly in the region between October 2005 and April 2007.

The results showed that the choice of a spouse having the same level of study is a frequent phenomenon. In fact, nearly one in three women has the same study level as her husband. Moreover, according to the survey results, more than one third of the female population marry men who resemble their fathers. Indeed, more than half the girls whose fathers are farmers marry farmers. Furthermore, individuals show a greater tendency to marry a spouse of the same social status.

P05 Prenatal and perinatal genetics

P05.01 Prenatally diagnosed two distinct cell lines of 18p terminal deletion/monosomy X possibly due to chimerism

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Monosomy 18p is a rare disorder with an incidence of about 1:50000 live born infants. Here we report a patient with mosaic 18p deletion accompanying to monosomy X. The case was the second live birth of a non-consanguineous couple. She was prenatally diagnosed by fetal blood sampling and amniocentesis at the 26th gestational week due to ultrasonographic findings of cleft lip/palate and polyhydramnios and the karyotype was 46,XX,del(18)(p11.2)/45,X. The parents decided to continue pregnancy and she was born at 36th gestational week. Her birth weight, length and head circumference was 2080 g (3-10p), 41 cm (<3p) and 32 cm (25-50p) respectively. Physical findings included asymmetric cranium, micro/retrognathia, low set ears, bilateral cleft lip, short neck and diastasis recti. Postnatal confirmation of karyotype from peripheral blood revealed 46,XX,del(18)(p11.2) in all 20 metaphases and subsequent FISH analysis for X aneuploidy resulted in 4.4% monosomy X mosaicism in 1255 cells showing the dominant cell line was 46,XX,del(18)(p11.2). Subtelomeric FISH analysis supported the 18p deletion. Cranial MRI, abdominal ultrasonography, ophthalmologic, oto-rhino-laryngologic and orthopedic examinations were normal. Pelvic ultrasonography for internal genitalia was planned but has not resulted yet. The mild clinical findings of our case except for cleft lip due to 18p deletion can be explained by the compensation of the 18p deletion by the presence of the cell line with 45,X karyotype and two distinct cell lines in this individual may be due to chimerism resulted from an embryonic fusion of twin pregnancy.

P05.02 The verification of ADAM12 effectiveness in first and second trimester aneuploidy screening

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The aim of the study was to verify ADAM12 aneuploidy screening effectiveness in the 1st and 2nd trimester.

The ADAM12 levels in frozen maternal sera from controls and from fetal aneuploidies were measured by DELFIA ADAM12 Research Kit (Perkin Elmer). For each day, in average 30 samples were used for control percentile (P) calculations. The 85 1st and 2nd trimester sera from pregnancies with autosomal and heterochromosomal aneuploidies were tested to ascertain the decrease under P25.

The P50 levels within week 9-19 rise from 254 to 1337 ng/ml with average daily increase of 15 ng/ml. The screening effectiveness between 1st and 2nd trimester was not different. Screening positivity (<P25) within week 9-19 was for T21 48 % (15/35), T18 57 % (4/7), T13 100 % (4/4), triploidy 89 % (8/9), 47,XXX 88 % (7/8) and for 45,X 12,5 % (1/8). The highest level of decrease from P25 was for T21 within range P10-25 (22 %). In other trisomies the highest positivity was within P1-P5: T18 29 %, T13 50 %, triploidy 78 % and 47,XXX 62,5 %. ADAM12 did not detect 47,XXY, 47,YYY and mosaic form of 45,X.

Our results indicate inverse relationship between the degree of phenotypic alteration in autosomal aneuploidies and decrease of ADAM12 under P25. It is remarkable, that the same is true for 47,XXX with mild phenotypic affection. Our results suggest, that ADAM12 is important marker for severe autosomal aneuploidies, including triploidy and 47,XXX in 1st and 2nd trimester.

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P05.03 Molecular study of alpha-globin non-deletional mutation in Iran.

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Introduction: Alpha thalassemia is the most common hemoglobin disorder in Iran. Most of known alpha thalassemia mutations include deletion of one or both alpha globin genes. However a considerable number of cases remain unknown after investigation for common deletion. In this case, investigating for point mutation in alpha-globin genes may help to detect the mutation and make a correct decision for performing prenatal diagnosis (PND) and differentiating unknown alpha thalassemia from normal HbA2 beta thalassemia.

Material and Methods: Couples referred to Kawsar Genomics Center for PND investigated for common deletional alpha-globin mutations (including - α 3.7kb, - α 4.2kb, -20.5 and -Med) using multiplex Gap-PCR. Those showed no deletion, further investigated for point mutation using direct sequencing using ABI-3130 genetic-analyzer.

Result: From 281 suspected cases with low MCV, low MCH and normal HbA2 and no common deletion which investigated using direct sequencing, 148 (52.67%) showed at least one point mutation either in α 1-globin or α 2-globin. More than 20 different mutation was detected. Most common mutation was Poly A2 (AATAAA>AATGAA) in 38 cases (25.67%) followed by Poly A1(AATAAA>ATAAAG) in 26 cases (17.57%) and CD142-CS (TAA>AAA) in 14 cases (9.46%). 121 cases (81.76%) showed a mutation in α 2-globin gene.

Conclusion: This study, showed using sequencing for further investigation of α 1-globin or α 2-globin genes can help us to increase accuracy of prenatal diagnosis for alpha thalassemia. Because of much higher frequency of mutation in α 2-globin, it seems it is much cost effective to sequence α 2-globin at first.

P05.04 The study of Alpha-thalassemia prevalence in referral patients to Pasteur Institute of Iran during September 2003 to September 2009

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Background and Objective: Alpha thalassemia is the most common inherited disorder of hemoglobin synthesis in the world. Unlike beta-thalassemia, in which nondeletional mutations predominate over 95% of recognized alpha-thalassemia involves deletion one or both alpha-globin genes. The aim of this study is the determine the prevalence of alpha-thalassemia frequency in provinces of Iran during September 2003 to September 2009.

Materials and Methods: The assay was tested on a total 446 blood and prenatal DNA samples. The samples were referred to genetic laboratory of Pasteur Institute of Iran from health centers. After obtaining informed consent, blood sample was taken at initial visit and genomic DNA was extracted according to salting out method. Multiplex Gap-PCR method and direct DNA sequencing were performed to detect alpha-globin gene deletions and nondeletional mutations, respectively. Results: Out of 446 samples 153 were carrier for deletions, 40 had nondeletional mutations and 253 were unknown. Among prevalence deletions the - α 3.7 (77.1%) is the most common alpha-thalassemia in Iran. The less common deletion was - α 20.5(6.5%), other deletions were, - α Med (8.5%) and - α 4.2 (7.9%). Nondeletional mutations were detected by direct DNA sequencing includes: PolyA(47.5%), -5nt(20%), Cd59(Hb Adana)(12.5%), Hb C.S, Cd19, Hb Q(15%), Cd21(Hb Fontainebleau), 11bp deletion(5%).

Discussion: MCV and MCH were <65-70 and <18-22, respectively. The study showed that in severe deletions (e.g. - α 20.5, - α Med) However, in mild deletional and nondeletional mutations (rest mutations), MCV and MCH were >70-73 and >21-24, respectively.

P05.05 Aneuploidy Detection by QF-PCR of STR Markers on the Applied Biosystems 3500xL Genetic Analyzer

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Analysis of short tandem repeat (STR) markers using quantitative fluorescence PCR (QF-PCR) is a common strategy employed in clinical research laboratories for the detection of chromosomal aneuploidy. Laboratories that routinely perform these types of analyses demand high-throughput, efficient, and highly automated solutions. Here we demonstrate performance equivalent between the Applied Biosystems 3130 Genetic Analyzer and the new Applied Biosystems 3500 Genetic Analyzer for the detection of aneuploidy using the Elucigene™ QST*Rplus assay. A large panel of cell-line and clinical samples were assessed to demonstrate the accuracy, ease of use, and throughput capabilities of the 3500 system for aneuploidy analysis of chromosomes 13, 18, 21, and sex chromosomes X and Y. The advanced capabilities of the 3500 Series Genetic Analyzers, including new thermal control systems, enhanced optical detection, and new consumables designs, provide an easy-to-use platform for the detection and analysis of multiplexed QF-PCR assays. The optional normalization reagent (GeneScan™ 600 LIZ® Size Standard v2.0) and compatible run module enable increased precision and accuracy in relative peak area or height determinations, which are particularly important for aneuploidy analysis. In addition, flexible GeneMapper® Software v4.1 was used to create reports and calculations to give user-configured tools for reporting multiplexed QF-PCR assay results. For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

P05.06 The values of echographic markers versus biochemical markers for antenatal screening of chromosomal aneuploidies

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At present, antenatal screening of the chromosomal aneuploidies includes evaluation of the echographic markers, biochemical markers and cytogenetic analysis using chorionic villus samples, amniotic fluid and blood from umbilical cord. Even if it is not a rule, frequently the prenatal cytogenetic analysis is request based on an abnormal ultrasound, biochemical markers or advanced maternal age. We present the cytogenetic analysis results of 210 cases of amniocentesis. These cases were evaluated as pregnancy at risk for chromosomal aneuploidies based on echography, biochemical markers or advanced maternal age and referred for cytogenetic analysis to our Genetics Laboratory. 63 cases were investigated for echographic signs suggestive for chromosomal aneuploidies: increased nuchal translucency, shortened femur length, echogenic bowel, choroid plexus cysts and echogenic intracardiac foci. In this lot we found one case of trisomy 18 and one case of monosomy X. Out of 82 cases with abnormal biochemical screening, one case was diagnosed with trisomy 21. Out of 57 patients with abnormal combined ultrasound and biochemical screening, one case was diagnosed as having trisomy 21 and one case with trisomy 13. The advanced maternal age was the reason for cytogenetic evaluation in 8 cases and one case of trisomy 21 was found. The ultrasound investigation was more reliable than biochemical screening for the detection of chromosomal aneuploidies. The risk was increased when two markers were abnormal (ultrasound and biochemical) and we would recommend karyotyping under these circumstances, the cytogenetic analysis remains the gold standard for the prenatal diagnosis.

P05.07 Clinical application of whole genome array-CGH during prenatal diagnosis: study of 25 selected pregnancies with apparently balanced structural aberrations or ultrasound findings

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The aim of this project was the application and evaluation of microarray Comparative Genomic Hybridization (array-CGH) in selected

cases during prenatal diagnosis.

Array-CGH was applied in 25 fetal samples with normal karyotypes and abnormal ultrasound findings, or with apparently balanced structural aberrations with or without ultrasound findings. 1Mb BAC array-CGH (Cytochip, BlueGnome Ltd.) was applied and abnormal results were confirmed with either Fluorescence In Situ Hybridization (FISH), Multiplex Ligation-dependant Probe Amplification (MLPA) or Real-Time PCR.

Three out of 25 samples (12%) referred for prenatal array-CGH carry copy number alterations. The rate of clinically significant alterations is 2/25 (8%) and the rate of findings with uncertain clinical significance is 1/25 (4%). Two benign CNVs were also found in 1/25 cases (4%).

The outcome of this study indicates the ability of array CGH to identify chromosomal abnormalities which cannot be detected during routine cytogenetic analysis, therefore increasing the overall detection rate of prenatal diagnosis.

P05.08 Axenfeld-Rieger and Dandy-Walker malformations in two fetuses with 6p subtelomeric deletion

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We report two unrelated fetuses with Axenfeld-Rieger malformation, and a 6p subtelomeric deletion. The first fetus had an unbalanced translocation inherited from its father : 46XY, der (6) t(6;8)(p23;p23) resulting in a partial monosomy 6p23-pter and a partial trisomy 8p23-pter. The second fetus had a de novo distal deletion of the short arm of chromosome 6 : 46XX, del (6p24.3-p25.3). Both had an Axenfeld-Rieger malformation (abnormal cleavage of the anterior chamber of the eye and hypoplasia of the iris) and Dandy-Walker malformation (enlarged cisterna magna and hypoplasia or agenesis of cerebellar vermis). Other anomalies included, for the first fetus, hypertelorism, ventricular septal defect, genital hypoplasia, diaphragmatic hernia and cochlear hypoplasia and for the second fetus, hypoplasia of the left heart and cleft lip and palate. Breakpoints were mapped by BAC-array CGH (Perkin). In both cases, the forkhead transcription factor gene *FOXC1* was deleted. The Axenfeld-Rieger malformation is a well known developmental anomaly of the eye, which occurs in as an isolated malformation or in a multiple congenital anomalies (MCA) context and related to mutations or deletions of a chromosomal region in 4q25 (*PITX2* gene) or in 6p25 (*FOXC1* gene). *FOXC1* has recently been shown to play a role in cerebellar development and the Dandy-Walker malformation (Aldinger *et al.*, 2009).

To our knowledge, this is the first fetal report of Axenfeld-Rieger malformation including a cochlear anomaly.

P05.09 Comparison of plasma cell-free DNA levels with gene expression profiles of peripheral blood cell during hemodialysis

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Objective: The levels of cell-free DNA (cfDNA) have been considered as potential marker of biocompatibility of membranes used for hemodialysis. Some studies demonstrated the elevations of cfDNA concentrations during the hemodialysis. Therefore we focused on comparison of plasma cfDNA levels and expression of selected genes in peripheral blood cells during the procedure. We selected genes with known functions in the process of inflammation and apoptosis.

Methods: The concentrations of plasma cell-free DNA of 30 patients undergoing hemodialysis (HD) have been measured and compared with the cfDNA levels of 30 healthy volunteers (before HD, after the first, second and third hour of HD and 30 min after HD). The mRNA expression of selected genes (pathways regulated by BAX, BCL-2 and by TNF receptor, caspase and interleukine families) were measured (immediately before and after HD) using real-time PCR technology. Plasma cfDNA was isolated and DNA quantification was carried out by

the real-time PCR method using GAPDH gene sequence.

Results: We found significant elevations of cfDNA in plasma during hemodialysis sessions with the highest values at the end of HD and with the rapid decline to normal levels within 30 minutes after HD in most patients. In comparison with healthy controls, the cfDNA levels in patients were significantly higher in the interdialytic interval. In expression profiles, we found individual differences among our patients and we discuss these differences with regard to the kinetics of cfDNA during HD session.

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P05.10 Non-invasive prenatal diagnosis for beta-thalassemia using pyrophosphorolysis-activated polymerization (PAP)

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Currently, prenatal diagnosis for hemoglobinopathies relies mainly on invasive testing, using chorionic villi sampling (CVS) or amniocentesis (AC). The risk of miscarriage following CVS or AC is 1-2%. The discovery of fetal cells and free fetal DNA (ffDNA) circulating in maternal plasma has led to the possibility of reliable non-invasive prenatal diagnosis (NIPD). Recently, a highly specific PCR technique was introduced, pyrophosphorolysis-activated polymerization (PAP), which has been shown successful in fetal sex determination using ffDNA.

Our aim is to develop a PAP-assay for NIPD of beta-thalassemia. Instead of designing PAP primers for each of the ~180 putative beta-thalassemia causing mutations and optimizing the conditions for each of them, we have chosen to select informative SNPs (i.e. with a frequency of >10% determined in a pilot study in 6 subsets of different populations) in linkage to the normal or mutant allele derived from father and absent in mother.

We have designed PAP-primers for 15 informative SNPs within the beta-globin gene cluster. After optimizing reaction conditions using genomic DNA, mixtures of wildtype and homozygous SNP samples were prepared to test the sensitivity of the assay. Until now, 16 PAP reactions for 10 SNPs are optimized and able to detect <3% target DNA in a background of >97% wildtype DNA. We have developed a primer for both wildtype and SNP variant for 6 SNPs. The next step is to retrospectively test plasma samples of pregnant women referred to our department for prenatal diagnosis using CVS or AC. The first results will be presented.

P05.11 BTK (Bruton Tyrosine Kinase) mutations in X-linked agammaglobulinaemia (XLA)

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Introduction: X-linked agammaglobulinaemia (XLA; MIM 300300) is an immunodeficiency characterized by failure to produce mature B lymphocyte and is associated with a failure of Ig heavy chain rearrangement. Affected males suffer from recurrent bacterial infections starting in early childhood. The immunoglobulin levels are reduced and B lymphocytes number is less than 1% of the normal level. The XLA is caused by BTK gene mutations. BTK gene is mapped on Xq21.3-q22 and codes for a cytoplasmatic tyrosine kinase.

Material and methods: Genomic DNA was extracted from 23 patients with XLA clinical diagnosis and 30 relatives. The 19 exons of BTK gene were amplified by PCR (Vorechovsky *et al.*, 1995). The amplicons were sequenced and analysed in an ABI Prism 310.

Results: 17 different mutations were detected, 6 missense (C154F, R288W, R288Q, V535F, D579V and R641H), 4 nonsense (K53X, R255X, Y361X and E441X) and 7 new mutations: 2 missense (K18R and T354I), 1 nonsense (E7X), 2 frameshift (H285X286, Y345X402), 1 splicing mutation (IVS7+1G>T) and 1 large undefined deletion (g.IVS15_IVS18del). So far, carrier status was detected in 18 out of 30 relatives. 2 PND were performed and the familiar mutation was absent

in both male foetuses.

Conclusions: The X-linked agammaglobulinaemia was confirmed by the identification of one BTK mutation in all cases studied. All families studied presented private mutations. Carriers could be identified and it is now possible to offer PGD and PND to these families. An early diagnosis of XLA will allow a correct clinical surveillance and an immunoglobulin replacement therapy.

P05.12 Non-invasive prenatal diagnostic MLPA test detects fetal Y-chromosome using cell-free fetal DNA extracted from maternal blood plasma early pregnancy

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Chorionic villus sampling (CVS) or amniocentesis (AC) is used to detect chromosomal abnormalities including trisomy 21. The major disadvantage herewith is the procedure-related risk of abortion. Currently, no cheap, non-invasive prenatal diagnostic test exists to detect chromosomal abnormalities. We aim at developing a targeted prenatal diagnostic screening test applicable in pregnancy using cell-free fetal DNA present in maternal blood plasma. In this study we used a "Multiplex Ligation-dependent Probe Amplification" (MLPA) P095 kit in order to detect chromosome 13, 18, 21, X and Y abnormalities. Therefore, peripheral blood plasma samples were collected from A. Women undergoing pregnancy termination because of trisomy 13, 18 or 21 detected with CVS (n=4) or AC (n=10), B. Women undergoing prenatal screening at 12-14 weeks of gestation with low risk (n=4), C. Women undergoing AC because of age \geq 36 years (n=3), and D. Non-pregnant control women who had 0,1,2 or 3 offspring (n=9). So far, all samples were tested with the MLPA P095 kit after specific amplification to detect fetal Y-chromosomal sequences. The MLPA test results obtained were compared with the CVS, AC or pregnancy outcome and controls. All but one false negative sample did correlate with the non-invasive MLPA test results, detecting the fetal Y-chromosome. Two out of three Y-MLPA-probe sets within the P095 kit were reliable. We conclude that further validation and quantification is needed to detect fetal Y chromosome and/or fetal trisomy 21 in non-invasively obtained cell free fetal DNA.

P05.13 Cell free fetal nucleic acid SNP quantitative analysis for potential noninvasive prenatal diagnosis purposes.

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Introduction: Genotyping based on the SNP analysis could be useful tool for detection and quantification of cell free fetal (cff) DNA/RNA in maternal plasma. For this purpose, is needed to choose genes according to their specific expression in placenta and chromosomal localization. Selected SNPs in particular genes should display high level of variability. Also, there is necessary to select more SNPs in several genes to ensure the cffDNA/RNA detection.

Aims: Our aims include: optimization and calibration of SNPs in cffDNA obtained from blood plasma of pregnant women, SNP genotyping in parents and fetuses followed by cffDNA SNP quantification of plasma cffDNA in pregnant women in the first and second trimester. Project is supported by IGA MZCR NS9624.

Method: We designed 12 SNPs *in silico* in following genes: DSCR4, PLAC4, KRTAP26, PLAC1, PAPPA, PSG11. Particular genotypes were previously determined by sequencing of SNP regions in 30 random samples of maternal DNA. Then, TaqMan RealTime PCR system in chosen SNP regions were optimized and calibrated. SNP quantification was made on cohort of 80 DNA samples isolated from plasma in

pregnant women.

Results: Heterozygosity in the SNPs varied from 14 % to 55 % with probability of the proper genotypes (Mother's SNP differs from fetal) detection from 12 % to 28 %. Sensitivity of minority genotype detection depends on the fluorescent background of non-specifically bound probe. The most sensitive TaqMan SNP systems were able to capture 1% artificial genotype mixture. Our analysis confirmed direct dependence of fetal DNA amount with the week of gestation.

P05.14 TNF α genotypes and risk for cerebral palsy in preterm and term infants

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Background. Cerebral palsy is a nonprogressive motor disorder caused by white matter damage in the developing brain. Activation of the cytokine network by a variety of insults (infection and/or hypoxia-ischemia) and elevated levels of proinflammatory cytokines can cause white matter brain damage as well as the development of CP. Recent data suggest a significant role of TNF α in the pathophysiology of perinatal brain injury. The aim of our study was to investigate the influence of TNF α promoter SNPs on susceptibility to CP in preterm and term neonates.

Methods. We analyzed 94 CP patients and control group of 118 unrelated children with comparable gestational age and sex distribution. Real-time PCR SNP analysis of TNF α SNPs was performed using pre-developed TaqMan SNP genotyping assay reagents.

Results. Statistically significant association between TNF α -1031 T/C high expression genotypes (TC and CC) and risk of CP was observed (OR 2.1811 (1.2075-3.9396), p=0.0097). Statistically significant association was also found between TNF α -1031C high expression allele and risk of CP (OR 1.9909 (1.1831-3.3501), p=0.0095). Statistically significant association between TNF α -1031 T/C high expression genotypes (TC and CC) and risk of CP was observed in term children (OR 4.7475 (1.5576-14.4696), p=0.0062) but not in preterm infants. Statistically significant association was also found between TNF α -1031C high expression allele and risk of CP in term children (OR 3.0952 (1.2124-7.9020), p=0.0181). CONCLUSION. Our results suggest a role of TNF α -1031 T/C gene polymorphism as modifying factor for development of CP in term, but not in preterm infants.

P05.15 Difficulties of interpretation of a complex chromosomal rearrangement in the prenatal period.

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Complex chromosomal rearrangements (CCR) are considered as rare events, but the frequency of their discovery increases with the contribution of molecular cytogenetic techniques. Here we report on the identification of apparently inherited balanced complex chromosomal rearrangement during prenatal period. It was the 4th pregnancy from healthy unrelated parents after 3 early miscarriages. First trimester was uneventful but second trimester was marked by the discovery of facial features during ultrasounds (right cleft of the lip and palate, flat fronto-nasal angle, hypertelorism). Fetal karyotype performed on amniotic fluid culture (AF) evoked an apparently balanced CCR involving the extremities of the long arms of chromosomes 2, 8 and 18. Paternal karyotype was normal but the mother had CCR resembling those found in the fetus. M-FISH on AF culture showed an unbalanced CCR (distal 8q trisomy, distal 18q monosomy). However, maternal blood M-FISH showed a more complex apparently balanced CCR with 4 breakpoints leading to an insertion of 8q material on 2q, a translocation of 2q material on 8q and a translocation of 8q material on 18q. Based on the maternal blood karyotype, we hypothesized a meiotic recombination event between the derivative chromosome 2 and the chromo-

some 8 in the fetus. Microarray confirmed the CCR with chromosomal breakpoints (8q23.3 and 18q21.33) and estimation of the sizes of the 18q deletion and 8q duplication. This CCR found during a pregnancy was *prima facie* an apparently balanced exchange between 3 chromosomes, but was the product of an unbalanced meiotic recombination from a maternal CCR.

P05.16 Reverse-hybridization teststrips for the detection of common CYP21A2 mutations in dried blood spots from newborns with elevated 17-OH progesterone

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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. CAH occurs in severe „classical“ forms (salt-wasting or simple-virilizing) or mild „nonclassical“ forms. The average incidence of classical CAH is about 1 in 15,000 births worldwide, and newborn screening programs based on 17-hydroxyprogesterone (17-OHP) levels have been introduced in various countries. Since neonatal 17-OHP screening has a considerable false positive recall rate, causing a substantial economical burden and emotional stress for parents, concurrent genetic testing for CYP21A2 defects to corroborate or exclude CAH diagnosis would be desirable.

We have developed a reverse-hybridization assay for rapid and simultaneous analysis of common CYP21A2 mutations from dried blood spots. The entire CYP21A2 gene is amplified in two overlapping fragments using PCR primers that will not co-amplify the highly homologous pseudogene. Biotinylated amplicons are hybridized under exactly defined stringency to a teststrip presenting a parallel array of allele-specific oligonucleotide probes for the following 11 mutations: P30L, IVS-2 A/C>G, 8 bp del (exon 3), I172N, I236N/V237E/M239K („cluster“), V281L, F306 insT, Q318X, R356W, P453S and R483P. Specifically bound PCR products are detected using enzymatic colour reaction.

The new CAH StripAssay is currently being validated on DNA samples of known CYP21A2 genotypes. Automated instrumentation and use of a scanner-based software tool (StripAssay Evaluator) for recording and interpreting results may further contribute to making the StripAssay a useful tool in CAH newborn screening programs. (oberkanins@viennalab.co.at)

P05.17 Role of genetic and iatrogenic factors in congenital hydronephrosis: clinical and therapeutic challenges in a romanian clinical setting

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Congenital hydronephrosis is a serious disease occurring among newborns, represented by the dilatation of the renal calyces associated with atrophic changes in the renal parenchyma resulting from obstruction of urinary flow, caused besides the intrinsic genetic factors by in utero exposure to certain drugs.

Our objective was to achieve a complete postnatal evaluation of patients identified prenatally with congenital hydronephrosis in a Romanian clinical setting.

In a two year longitudinal study, from January 2007 to December 2009, we prospectively enrolled and followed up 26 women whose fetuses were detected with hydronephrosis. Prenatal diagnosis of hydronephrosis was achieved by echography; affected children were followed-up and evaluated after six month.

Results: 9 women (34.61%) of the cases reported hydronephrosis in two (77.77%) and even three (22.22%) consecutive generations. In 17 cases of birth outcomes (65.38%), expectant mothers were exposed to different drugs in the first trimester of pregnancy: analgesic drugs - 15 cases (88.24%) and respectively antitussive agents (dextrometho-

rphan) in 2 cases (11.76%).

These findings illustrate the complex decision-making process when coordinating the antenatal identification with the appropriate postnatal evaluation and treatment for each pediatric patient.

P05.18 Monitoring of Congenital Malformations and Efficacy of Prenatal Diagnostics in Tomsk Region

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The results of monitoring of congenital malformations in Tomsk during nine-year period since 1998-2007 and value of efficacy of prenatal diagnostics of some forms of congenital malformations has been analyzed. Frequency of prenatally detected and interrupted congenital malformations during period studied varied from 0,6 to 6,3 per thousand, the malformations of central nervous system made basic contribution to this index. There was significant decrease in frequency of some malformations during the period studied, with negative correlation between year of observation and malformation frequency (transposition of the great vessels, $r = -0,86$; diaphragmatic hernia, $r = -0,78$; omphalocele, $r = -0,61$; birth defects of central nervous system, $r = -0,61$; and Down syndrome, $r = -0,53$). Dependence of reduction in frequency of spina bifida and Down syndrome among newborns was determined from frequency of induced abortions of fetuses with these congenital malformations. Frequency of these forms of birth defects among newborns significantly decreased together with increasing of frequency of the same forms among induced abortions ($r = -0,64$). So, this dependence may testify for effective measures of prenatal diagnostics in decreasing of level of congenital malformations among newborns. Apparently, improvement of preventive system of congenital malformations may significantly decrease the frequency of birth defects among newborns.

P05.19 Oxidative stress and sperm DNA Fragmentation-Paternal role in recurrent spontaneous abortions.

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Introduction: Paternal genome integrity is important in initiation and sustenance of viable pregnancy in both natural and assisted conceptions. Nicks/breaks and/or nucleotide modification caused by unregulated free radical concentration due to inefficient antioxidant machinery leads to sperm DNA damage. So antioxidant capacity assay and sperm DNA integrity assessment are imperative in analysis of pregnancy loss.

Material and Methods: Male partners of 77 couples [Group A (n=41) spontaneous conceptions; Group B (n=36) assisted conceptions] experiencing recurrent pregnancy loss and 41 fertile controls were included in study. Total antioxidant capacity (TAC) of seminal plasma was assessed by commercially available kits. Sperm DNA integrity was quantified by single cell gel electrophoresis (comet assay). Mann-Whitney test was applied for statistical significance.

Results: Seminal TAC was reduced in patients ($p=0.0017$, $p=0.003$ in group A and B respectively) as compared to controls. Higher level of DNA damage (number of comet with increased tail length) were observed in both group of infertile men as compared to fertile controls ($p=0.005$ and $p=0.007$ in group A and B respectively).

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. Thus oxidative stress assessment and DNA integrity analysis should be undertaken in all couples experiencing recurrent spontaneous abortions.

P05.20 Prenatal diagnosis of Donohue syndrome (Leprechaunism)

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Donohue syndrome (OMIM: 246200) is a severe congenital insulin-resistance syndrome described in 1954 that leads to death, often in early infancy. It is caused by mutations in the insulin receptor gene *INSR* (19p13.3-p13.2) and it is inherited as an autosomal recessive condition. Prevalence is estimated to be 1 in 1 000 000 live births.

We present a prenatal diagnosis of *Leprechaunism* performed in Spain in a non-consanguineous couple with a previous affected son. This patient was born after a 41 week pregnancy and showed intrauterine growth retardation detected from the sixth month. He died at 18 months due to a respiratory failure. The mutations identified were p. R1026X inherited from the mother and IVS19+5 g>a (p.D1176GfsX8) from the father. Both predict null alleles and were not previously described.

Molecular diagnosis of the new gestation was made at 11+6 weeks gestation in a sample of chorionic villus by automated sequencing of exon 17 and intron 19.

The result was a female foetus with no mutation inherited, being the first prenatal diagnosis made in our population.

P05.21 Prenatal diagnosis of an autosomal translocation with regular Trisomy 21

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The coincidence of trisomy 21 (regular Down syndrome) and a structural rearrangement is very rare. Even, it is not reported as a prenatal diagnosis. In this report we present an autosomal translocation carrier fetus with trisomy 21: 47,XX,+21,t(3;8)(p21;q24). The coexistence of reciprocal translocation and trisomy may be seen in reciprocal translocation carrier families, de novo cases are extremely rare. The presented case is diagnosed by amniocentesis which is done due to abnormal fetal ultrasonographic findings and increased trisomy 21 risk at maternal serum screening. The postmortem pathologic examination of the fetus revealed novel findings associated with the breakpoints 3p21 and 8q24.

P05.22 The effects of Paracetamol and Ketonal administration, during embryogenesis, over the conception product

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Aims: To highlight the possible occurrence of congenital malformations following the administration of chemical substances during embryogenesis, by conducting an experiment on animal model.

Material and method: The experiment took place during 15.05.2006 and 30.06.2006 in the Laparoscopic and Microsurgery Center, UMF Timisoara, on a group of 20 pregnant female white mice. After weighing, in hypogastric region was given a dose depending on weight. The procedure was repeated for each animal 3 days consecutively. At the end of pregnancy the born fetuses were examined. After birth, the intranatal exposed group was euthanized and macroscopic and microscopic examination was made on liver and kidney, compared to a control group.

Results and discussion: Regarding the fertility of intranatal exposed animals, they had babies in a species characteristic number, which were apparently normal. In terms of macroscopic examination of the liver and kidneys, the presence of anomalies was not highlighted. Microscopic examination indicated a later suffering liver and kidney in animals exposed to the intrauterine action of paracetamol and ketonal.

Conclusions: Administration of paracetamol and ketonal during pregnancy in mice did not influence the evolution of pregnancy. The resulted mice have had a normal morphological development compared with control group. Microscopic examination of liver and kidney indicated the presence of pathological changes.

P05.23 A familial t(15;22)(q13;q11.1), implications for prenatal genetic testing

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Pregnancy in a familial balanced translocation carrier can pose a challenge to a genetics testing laboratory, particularly when the rearrangement is G-band subtle and involves chromosome 15. We report a t(15;22)(q13;q11.1) familial translocation that while easily distinguishable at the 600 band resolution could appear to be subtle at lower resolution. The mother, a phenotypically normal carrier of the translocation, reported a family history of a Prader-Willi-like syndrome. To help confirm the breakpoints of the translocation, FISH analyses using a range of commercially available probes was undertaken, these demonstrated that 15q breakpoint was distal to SNRPN, while the 22q breakpoint was in the alpha-satellite region of 22q11. This therefore, resulted in the derivative chromosome 15 containing both SNRPN and the 22q11.2 region associated with Di George syndrome. At gestational week 11 a CVS biopsy was taken and sent to us for cytogenetic, MLPA and UPD studies. Due to the prior FISH testing, cytogenetic analysis could confidently define the foetal karyotype as 46,XY+der(15)t(15;22)(q13;q11.1),-22, thus resulting in partial trisomy of 15pter-15q13 and insignificant monosomy of 22pter-22q11.1. Both the MLPA and UPD studies were consistent with this finding. These results illustrate the importance of prior planning for prenatal testing of an individual with a family history of a balanced chromosomal rearrangement and the identification of the appropriate tests and markers.

P05.24 Non-invasive Bovine Fetus Sex Determination Using Multiplex PCR Analysis of Fetal DNA in Maternal Plasma

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Background: In order to establish a reliable non-invasive method for bovine fetal sex determination in routine setting the possibility of identifying the fetal X and Y-chromosomes specific sequences has been evaluated in maternal plasma using conventional multiplex PCR analysis. The aim of this study was to provide a rapid and reliable method for sexing bovine fetuses. **Methods:** Peripheral blood samples were taken from 38 pregnant heifer with gestational weeks of 12 to 38. DNA template was extracted by phenol-chloroform method from 350 µl maternal plasma. Two primer pairs for bovine amelogenin gene (bAML) and BC1.2 were used to amplify fragments from X and Y chromosomes. A multiplex PCR reaction has been optimized for amplification of 467 bp and 341 bp produced from X and Y bAML gene respectively and a 190 bp fragment from BC1.2 related to Y chromosome. **Results:** The 467 bp fragment was detected in all 38 samples and 341 and 190 bp fragments were detected only in 24 plasma samples that delivered a male calf. The sensitivity and specificity of test was 100% with no false negative and false positive results. **Conclusions:** The results showed that phenol-chloroform method is a simple and sensational for isolation fetal DNA in maternal plasma. The multiplex PCR is a available non-invasive methods which is cost efficient and replicable and is easily for bovine fetal sexing.

P05.25 Fetal RHD genotyping from maternal plasma: Lyonnaise study on 196 patients

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RHD genotyping from maternal plasma allows to optimize the care of pregnant women anti-D allo-immunized and to target prevention through Ig anti-D currently recommended by the Collège National des Gynéco-obstétriciens Français during the second trimester of pregnancy.

Exons 4, 5 and 10 of RHD gene are analyzed by real-time PCR using the TaqMan technology (Minon et al 2005).

DNA is extracted manually with the QIAamp kit (Qiagen ®) and then amplified on the real-time PCR instrument Lightcycler 480 (Roche ®). After validation of witnesses (including SRY and CCR5 genes), we analyze the correlation of cycle Threshold obtained for the 3 exons and compare them to the phenotype achieved at birth.

The RHD gene is present in fetuses where at least 1 of the 2 Ct obtained for each exon is positive. Any discrepancy between the exons implies, after verification of results, analysis of gene RHD on maternal buffy coat. Finally, each negative result found for the first time will be confirmed on another sample a few weeks later. A female fetus without RHD gene is characterized by the absence of amplification of the 3 exons and the SRY gene control.

For 195 tests, the results show a perfect correlation with the phenotype of RH1 newborn regardless of gender.

On 1 sample, we have demonstrated the presence of an RHD pseudogene.

The use of a gene control, hyper-methylated in the fetus, currently under study in our lab, would get rid of the SRY gene control, depending on the sex of the fetus.

P05.26 Quantitative analysis of foetal DNA in maternal circulation in anaemic pregnant women

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Advances in molecular genetics have allowed the investigation of the foetal genome through analysis of circulating foetal DNA in maternal plasma. Cell-free foetal DNA (fDNA) in maternal plasma or serum is widely investigated as a source of foetal genetic materials. Increased amount of circulating fDNA in maternal plasma has been found in some adverse pregnancies. It was suggested that elevation of fDNA in maternal plasma could be used for early identification of adverse pregnancies. To date, no study has been done to investigate the fDNA in anaemia, considered as the more common pregnancy related complication. The aim of this study was to quantify circulating fDNA levels in normal healthy pregnant individuals and pregnant women with anaemia. In this study, fifty seven samples consisting of anaemia (n=19) and normal pregnant women (n=38) carrying singleton male foetuses, were collected. The fDNA concentrations were measured by quantitative real-time PCR amplification using TaqMan dual labelled probe system. The SRY gene was used as a unique foetal marker. The mean fDNA concentration for normal pregnancy samples and anaemic pregnancy samples were 41.14 GE/ml and 30.96 GE/ml respectively. No significant differences were observed in the mean fDNA concentration between normal and anaemic pregnancy samples ($P=0.535$). In conclusion, anaemia does not significantly affect levels of fDNA in maternal plasma. Hence, if fDNA is used as an additional marker in prenatal screening test in the future, the findings of this study suggests that fDNA quantity will not be much informative for anaemic pregnancies.

P05.27 Founder mutations in the Tunisian Population: Implications for diagnosis in North Africa and Middle East

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Tunisia is a North African country of 10 million inhabitants at the cross road between Europe and Africa. Throughout its history, it has been the seat of the invasion and immigration of different ethnic groups. As its neighbouring and Middle Eastern countries, its population shows a high prevalence of consanguinity and endogamy, thus leading to emergence of genetic disorders at higher rates. Many factors could contribute to the recurrence of monogenic morbid trait expression. Among these, founder mutations that arise in one ancestral individual and diffuse through the population in isolated communities. By a review of the literature, data from PubMed and other sources including conference proceedings, two classes of founder mutations have been identified in the Tunisian population. The first includes founder mutations that are reported so far only among Tunisian patients, they are mainly the result of the high rate of consanguinity and endogamy. The second founder mutations are shared with other populations originating mainly from other North African or Middle Eastern countries and in certain cases from both shores of Mediterranean. These mutations have captured historical events in the region and are particularly useful for the development of easy and cost effective tools for molecular diagnosis.

P05.28 A new case of microdeletion of the FOX gene cluster on 16q24.1 in a preterm male infant: confirmation of the severity of pulmonary distress

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Genomic and genic deletions of the FOX gene cluster on 16p24.1 have been recently described in the context of alveolar capillary dysplasia, a rare lethal developmental anomaly of the lung, and other malformations resembling VACTERL association. We report the case of a preterm male infant, born at 26th weeks of gestation and deceased 16 hours after birth. During pregnancy, a cardiac malformation and bilateral hydronephrosis were observed at 19th AW. Karyotype analysis was normal and a 22q11 microdeletion was excluded by FISH analysis. A Cesarean section was performed at 26th AW due to fetal distress. Following delivery he presented a refractory hypoxemia. An autopsy was performed. Growth parameters were appropriate for gestational age. The heart showed partial AV canal malformation. Both kidneys showed dilatation of the pelvocaliceal system with bilateral ureteral stenosis. There was an annular pancreas. Microscopic examination of the lungs showed the characteristic features of alveolar capillary dysplasia with misalignment of pulmonary veins. Some features were less prominent due to the gestational age. Array-CGH analysis (Agilent oligoNT 44K) was performed on DNA extracted from uncultured fibroblasts, showing an interstitial microdeletion encompassing the FOX gene cluster in 16q24.1. The size of the deletion is 1.57 to 1.76 Mb. Complementary analysis using FISH and high-resolution array-CGH are currently ongoing to confirm a possible somatic mosaicism. We confirm the early onset and the severity of pulmonary distress associated with 16q24.1 microdeletions. This case also illustrates the interest of array-CGH analysis in prenatal diagnosis in case of fetal malformations.

P05.29 A pilot study of Fragile X carrier screening in China

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CGG repeat expansion in the 5' noncoding region of the *FMR1* gene on the X chromosome is associated with an array of disorders. Large expansion of over 200 repeats (full mutation) gives rise to the most common form of inherited mental retardation known as Fragile X Syndrome (FXS), whereas smaller expansion of 55-200 repeats (premutation) is recently shown to be involved in autism, premature ovarian failure, and fragile X-associated tremor/ataxia syndrome (FXTAS). More importantly, the expanded CGG repeats are unstable and can undergo further expansion on female transmission. Thus, screening for the unstable *FMR1* alleles in asymptomatic women before or at early pregnancy is an efficient strategy for the prevention of the related diseases. To evaluate the feasibility of a Fragile X carrier screening program in China, we anonymously examined 935 Chinese women from our obstetric clinic for the *FMR1* CGG repeats status. DNA analysis was carried out by an enhanced PCR, coupled with capillary electrophoresis separation. Inconclusive results were subsequently verified by Southern analysis. The results showed that 72.5% of the samples were heterozygous for the *FMR1* alleles, 29 and 30 CGG repeats were the most common alleles. In total, we identified 1 premutation (55-200 CGG repeats) and 9 intermediate expansions (45-54 CGG repeats), no full mutation was found. The incidence of premutation (1:935) and intermediate expansion (1:104) in this cohort is comparable to that reported in Caucasian population. Our finding indicates that implementing a Fragile X carrier screening program may be practical in Chinese population.

P05.30 Non-Invasive Prenatal Diagnosis on Plasma DNA from RHD Negative Pregnant Women with Free DNA Fetal Kit RhD®

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Hemolytic disease of the newborn is the clinical condition in which couples Rh blood group antigens are incompatible with each other and mother is negative for the antigen whereas father is positive. Fetal cells circulate in maternal peripheral blood. The possibility of analyzing fetal cells recovered from maternal plasma could provide high sensitivity prenatal diagnosis. In this study using a quantitative real-time PCR assay, the presence of RhD gene sequences was evaluated in the serum of patients at the onset of pregnancy. For each fetal RhD genotyping analysis DNA is extracted from maternal plasma. The presence of fetal RhD gene in the plasma DNA is detected by real-time PCR amplification of two different segments of the RhD gene (exon 7 and 10). Each amplicon is revealed with specific taqman probes. Our current findings accuracy of fetal RhD genotyping on maternal plasma using a Free DNA Fetal Kit RhD®

P05.31 Gene expression microarray analysis of amniotic fluid cells with trisomy 21

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Trisomy 21 is the most common cause of mental retardation, although the clinical picture is highly variable. The underlying mechanisms for the variability in phenotype remain to be determined.

Our hypothesis was that extra copy of chromosome 21 disrupts the normal profile of gene expression that alters the normal development of fetuses with trisomy 21.

We used Agilent 4x44K whole human genome microarrays to identify the measure of differences in gene expression variance between total RNA from 10 samples with trisomy 21 and 9 samples of euploid amniocytes matched with the reference RNA pooled from all above mentioned samples.

Analysis of data from microarray experiments has revealed significant differences in expression levels of 136 probes with p-values adjusted for multiple testing by Benjamini-Hochberg method estimated under 0.01. This differentially expressed set of probes defined 72 genes up-regulated and 54 genes downregulated in samples with trisomy 21. We compared our findings to the results previously reported by two studies available at the NCBI GEO repository with accession numbers: GSE6283 and GSE10758. Cross-study comparison identified 8 consistently significantly altered genes in the intersection of our results with the GSE6283 study. This set of candidate genes may represent a potential set of biomarkers for DS and will be further tested on independent samples of fetal trisomy 21, to evaluate the prediction value of the biomarker set.

P05.32 Should newborn screening be introduced for Pompe disease? Preliminary report on valuation of benefits and risks by the general public in the Netherlands

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Background: Enzyme replacement therapy is now available for Pompe disease. Across the broad phenotypic spectrum of this disease, the better the initial condition of the patient, the greater the effect of therapy. The rapidly progressive, infantile form of Pompe disease is currently diagnosed at a median age of 5 months. To shorten diagnostic delay, newborn screening is under consideration.

Primary screening of blood spots is sensitive for Pompe disease, but additional clinical and laboratory tests are needed to confirm the diagnosis and to distinguish severely affected infantile patients, who require immediate treatment, from infants who may go on to develop more slowly progressive symptoms, later in childhood or adulthood. The need for additional tests means that parents would not be able to opt out of learning a diagnosis of 'probable later onset disease'.

Aim and method: Early prediction of later-onset disease is not a goal of newborn screening, but if it were an inevitable by-product, as in the case of Pompe disease, policy-makers and governments must weigh

the consequences of screening for patients of all phenotypes. This study aims to measure the opinion of citizens on this issue, including valuation of benefits and harms as well as moral reasoning.

A novel, quantitative questionnaire was developed, including background information and scenarios of various screening outcomes. After pre-testing it has been submitted to a 1500-member consumer panel as well as to (parents of) patients with Pompe disease. Descriptive statistics and regression analysis of the survey results will be presented.

P05.33 Health and functional status of patients symptomatically diagnosed with Pompe disease .A case for earlier diagnosis through newborn screening?

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Background: Since enzyme replacement therapy for Pompe patients is most effective when started early and diagnostic methods on dried blood spots are improving, attention for newborn screening for Pompe disease is increasing.

Aim: Our study aims to depict the health and functional status of Pompe patients at the time of symptomatic diagnosis in order to illustrate the gains that might be achieved by early diagnosis via newborn screening. The outcome of this study may assist policy-makers in their consideration of newborn screening for Pompe disease.

Methods: Previously collected clinical data and results of the IPA/Erasmus MC Pompe Survey, an international patient oriented questionnaire study, were used. Cross-sectional data of 82 Pompe patients (age 0-62) were re-analysed to give a "snapshot" of their health status at time of symptomatic diagnosis. Following the ICF classification, we selected the following domains: body function and structure, activity, participation and contextual factors, taking into account that some damage might be irreversible.

Results: Around time of diagnosis of patients with classic-infantile Pompe disease, heart function, hearing, and muscle development and strength are severely impaired in most if not all cases. Use of oxygen and/or nasogastric tube-feeding was reported in almost 50% of these cases.

In juvenile and adult patients, primarily muscle strength and respiratory function are impaired at the time of diagnosis. The majority of juvenile and adult patients have some form of handicap. About 15% use a walking device and/or respiratory support by the time diagnosis is made. A more detailed description will be presented.

P05.34 Maternal of Glutathione-S-Transferase Genes Polymorphisms with Placental Insufficiency and Fetal Growth Retardation

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Placental insufficiency (PLI) is a key problem of obstetrics. The purpose of research is to study polymorphic alleles of glutathione-S-transferase genes (GSTT1, GSTM1, GSTP1) at PLI. GST-genes code xenobiotic-conjugating enzymes of phase II detoxication system.

Material and methods: 142 pregnant women were surveyed. PCR/RFLP was used for analysis of deletion genes GSTT1, GSTM1 frequencies and polymorphism of GSTP1 at PLI and control group. Genotype GSTP1 D includes genotypes A/B, A/C, B/B, B/C, C/C.

Results: The frequency of deletions genotypes of GSTT1 or GSTM1 is significantly increased in the group with placental insufficiency (53%) in comparison with control group (15%). In the group with fetal hypoxia the GSTP1 A/C genotype was revealed statistically more often (P=0,01), and at intrauterine growth retardation - GSTP1 D genotype (p=0,015). The risk of PLI development with the GSTM1 +/GSTT1+ GSTP1 A/A genotype is 11 times lower (OR 0,09, 95 % CI 0,04-0,019). The risk of development of fetal growth retardation with GSTM1 +/GSTT1 + GSTP1 D genotype is 5 times higher (OR 5,37, 95 % CI 1,05-27,5).

Genotype GSTP1 A/A has a high level glutathione-S-transpherase ac-

tivity of placenta ($p=0.029$). Genotype GSTP1, which carries alleles B and/or C, glutathione-S-transpherase activity of placenta was decreased ($ZT=2.37$, $p=0.018$). Glutathione-S-transpherase activity of placenta doesn't depend on genotypes GSTM1 and GSTT1. Carrying functionally weak alleles GSTP1 D in combination with active smoking during pregnancy provokes development of fetus growth retardation ($\beta_i=82.7$, $\chi^2=8.7$, $p=0.013$), in consequence of decrease of glutathione-dependant antioxidant detoxication in placenta.

P05.35 Expression of hsa miRNA 35 in normotensive, preeclamptic and HELLP syndrome patients

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Objective: The aim of our study was to examine whether the expression of micro RNA 325 (miRNA 325) differs in preeclamptic, HELLP syndrome and patients with uncomplicated pregnancy. Study Design: Micro RNA was isolated from placenta tissue samples obtained from 16 preeclamptic, 10 HELLP syndrome and 18 normotensive pregnant women. Micro RNA 325 was measured by real-time quantitative PCR. Results: Expression of miRNA325 are significantly elevated in uncomplicated pregnancies (median: 11.395 vs. 32.460 and 0.001 vs. 0.086 pg/ μ l; $P < .001$), and decreased in HELLP syndrome. The expression of miRNA325 was significantly lower in case of preeclampsia compared with HELLP syndrome, and normotensive patients. Expression was significantly higher in case of HELLP syndrome compared with normotensive patients. Conclusion: The expression of miRNA325 is downregulated in case of preeclampsia.

P05.36 Hydrocephaly/anencephaly-polydactyly in four siblings: a new locus HYLS2 on chromosome 15

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Hydrocephalus syndrome (HYLS, MIM236680) is a lethal polymalformative autosomal recessive disorder. It is characterized by the association of postaxial polydactyly of the hands and preaxial polydactyly of the feet, micrognathia and central nervous system anomalies which are often represented by hydrocephaly with absent midline structures and keyhole-shaped defect in the occipital bone. The majority of fetuses have been described from Finland with a prevalence estimated at 1/20000 birth in this population. A recurrent D211G mutation in HYLS1 is responsible of the Finnish HYLS cases, with a founder effect. To date, no other HYLS1 mutation has been found in non-Finnish HYLS cases.

Recently, the HYLS1 ortholog in *C. elegans* and *Xenopus* was shown to be necessary for the apical anchoring of centrioles at the plasma membrane, phenomenon which is required for ciliary formation. Therefore, HYLS has joined the group of ciliopathies despite the absence of renal abnormality.

Here we report 4 fetuses born from a consanguineous Algerian couple with a polymalformative syndrome associating hydrocephaly / anencephaly and polydactyly suggestive of HYLS without mutation in the HYLS1 gene. Homozygosity mapping using SNP chips Affymetrix 250K identified a new locus in this family which we define as the HYLS2. Further studies are under way to identify the second gene responsible for HYLS. A particular attention is given to genes involved in cilia biogenesis.

P05.37 Analysis of MTHFR C677T and A1298C polymorphisms in embryonic tissues from spontaneous abortions

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Introduction. Methylenetetrahydrofolate reductase (MTHFR) gene C677T and A1298C polymorphisms influence homocysteine metabo-

lism which in turn may contribute to the development of neural tube defects and unexplained, recurrent embryo losses in early pregnancy. In this study we have analyzed the MTHFR C677T and A1298C genotype distributions and their associations with compromised fetal viability with regard to the presence of aneuploidy.

Methods. In addition to cytogenetic analysis, DNA was extracted from spontaneously aborted embryonic tissues and adult controls. Analysis included detection of C677T and A1298C mutations with allele specific PCR method and molecular karyotyping with multiplex ligation-dependent probe amplification (MLPA).

Results. Comparison of combined MTHFR C677T/A1298C genotype distributions between spontaneously aborted embryos, normal and abnormal karyotype groups and controls is presented in table.

Conclusions. The present finding indicates decreased viability among fetuses carrying MTHFR mutations and a possible selection disadvantage among fetuses with increased numbers of mutant MTHFR alleles. Spontaneously aborted embryos have a unique distribution of MTHFR C677T and A1298C polymorphisms and their appearance is not related to chromosomal integrity in the abortus. Our final conclusion is that combined common polymorphisms of MTHFR play a role in fetal demise. However, the present finding of high prevalence of mutated MTHFR genotypes in spontaneously aborted embryos further emphasizes the clinical and biological significance of this gene in foetal development.

MTHFR C677T/A1298C genotype distributions

| GENOTYPE MTHFR C677T/A1298C | ABORTIONS (n=333) (%) | NORMAL KARYOTYPE (n=222) (%) | ABNORMAL KARYOTYPE (n=111) (%) | CONTROLS (n=100) (%) |
|--|--------------------------|------------------------------------|--------------------------------------|-------------------------|
| CC/AA | 48 (14.4) | 31 (14.0) | 17 (15.3) | 19 (19.0) |
| CC/AC | 30 (9.0) | 20 (9.0) | 10 (9.0) | 13 (13.0) |
| CC/CC | 26 (7.8) | 18 (8.1) | 8 (7.2) | 10 (10.0) |
| CT/AA | 51 (15.3) | 37 (16.7) | 14 (12.6) | 23 (23.0) |
| CT/AC | 53 (15.9) | 33 (14.9) | 20 (18.0) | 16 (16.0) |
| CT/CC | 23 (6.9) | 14 (6.3) | 9 (8.1) | 3 (3.0) |
| TT/AA | 41 (12.3) | 29 (13.1) | 12 (10.8) | 15 (15.0) |
| TT/AC | 38 (11.4) | 26 (11.7) | 12 (10.8) | 1 (1.0) |
| TT/CC | 23 (6.9) | 14 (6.3) | 9 (8.1) | 1 (1.0) |
| chi square (all samples vs. controls) | 46.64, P<0.001 (df= 8) | | | |
| chi square (normal karyotype vs. aneuploidy) | 10.83, p=0.21 (df= 8) | | | |

P05.38 Approach to the study of abortions with isolated limb malformation or as part of multiple congenital anomalies.

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BACKGROUND: Congenital limb malformation is clinically heterogeneous and may manifest itself as an isolated feature or in association with a particular syndrome. Providing accurate clinical diagnosis of abortions is an arduous task due to the insufficient information obtained from ultrasound studies or the absence of anatomical and pathological exams.

PATIENTS AND METHODS: 15 out of a total 500 abortions referred to our service because of limb malformation either with or without other multiple congenital anomalies, were selected. Karyotype, QF-PCR, MLPA (subtelomeric regions and genes involved in limb malformations) testing was performed. 7 out of 15 selected DNA samples were analysed with the commercially available Agilent 400k microarray.

RESULTS: Numerical chromosomal aberrations were detected in 8 cases either by Karyotype, QF-PCR or MLPA analysis. Agilent 400k microarray analysis showed significant genomic alterations in 5 of the 7 remaining cases, involving microdeletions and microduplications of regions containing genes related to limb malformation. One sample was degraded and in the other one non-pathological alterations were found.

DISCUSSION: Our results support the discarding of numerical chromosomal anomalies as the first step in the study of abortions with limb malformations. Microarrays assays are required for the cases in which chromosomal aberration is not the cause of the phenotype. In conclusion, both the selection process and the proposed strategy were suitable since genomic alterations were found in nearly all the sam-

ples. Nevertheless, exhaustive clinical information and further familiar molecular studies are essential to establish a more accurate genetic diagnosis.

P05.39 Determining the fetal gender and Rhesus D status by noninvasive prenatal method - a new approach in Romanian prenatal diagnosis field

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The prenatal diagnosis procedures currently employed in Romania are based exclusively on invasive sampling procedures of fetal cells. The discovery of circulating free fetal DNA in maternal plasma has provided an alternative to invasive procedures. Our aim was to investigate the possibility of using this approach to determine fetal gender and Rhesus D status.

We performed a short study: ten healthy pregnant women were included. All ten were RhD negative with RhD positive partners. Seven of them were carrying male fetuses. As controls, blood samples from RhD negative and positive healthy non-pregnant women and healthy males were analyzed. A peripheral blood sample was collected from each subject; the plasma was separated by a two-steps centrifugation method. The total free cell DNA was extracted using the QIAamp® DSP Virus kit according to the SAFE optimized protocol (Legler et al.). We determined the fetal gender and RhD status using both conventional and Real-Time PCR by detecting the fetus-specific Y (SRY and DYS14 genes) and the RHD gene (exon 7) sequences respectively. We monitored the β-globin as a housekeeping gene to determine the DNA extraction efficiency.

Conclusions: this non-invasive approach of fetal gender determination can be used as a screening procedure in cases at risk for inheriting an X-linked recessive disorder; the RHD gene detection can eliminate the unnecessary administration of anti-D immunoglobulin to pregnant women with RhD negative fetuses. We are able to detect fetal DNA in maternal plasma, with the possibility of extending this study to single gene disorder detection.

P05.40 Implementation of One-Stop-Clinic for Risk Assessment of chromosomal abnormalities in the first trimester in the Czech Republic (OSCAR)

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Prenatal diagnosis encompasses all measures of disease prevention from mass screening programs and use of new technologies to organizational and support systems used in the delivery of health care. The unwillingness to depart from old and tested methods can prevent an introduction of new methods, even though they are proven to be superior to the old ones.

Czech doctors are free to select technologies for diagnosing and treating patients. The selection is usually based on recommendations published by medical societies. The most practiced strategy for prenatal screening is a triple test (TT). Women with a positive TT and those over 35 years of age undergo amniocentesis with a possible late termination of pregnancy.

We present our experience in shifting prenatal screening of aneuploidies from 2nd to the 1st trimester in a large university hospital and a large regional hospital in the Czech Republic. Our decision to change the method of screening was based on the published data and a one year pilot study which we run at the department. A minimum investment was made to purchase the software (Astraia) and a blood analyzer (Kryptor). We started to book pregnant women for ultrasound examination in the first trimester and substituted triple test with first trimester biochemical screening. Anomaly scan including screening of the heart was organized for 20-22 weeks of pregnancy. For women who screened positive we immediately progressed to CVS and used PCR for the diagnosis of aneuploidies, with results available in less than 36 hours. Confirmed pathological cases were terminated according to the wishes of the women.

We started the clinic in January 2004 in the University Hospital (Olomouc) and in October 2007 in the Regional Hospital (Zlin). So far we screened about 18.000 pregnancies and detected 51 cases of trisomy

21 and 67 cases of other chromosomal abnormalities. Our false positive rate is 3,5% and detection rate for trisomy 21 is 98%.

Shifting the strategy of prenatal screening required only minimum investment in terms of money, and some reorganization of existing resources including manpower. The new screening method is very well accepted by women and compares very favorably with existing screening methods which have much lower detection rates and much higher false positive rate.

P05.41 Birth of a healthy baby boy after microsatellite PCR-based preimplantation genetic diagnosis of a reciprocal chromosomal translocation

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Carriers of balanced chromosome translocations usually do not show any adverse phenotypic effects other than experiencing difficulty establishing and maintaining a pregnancy, due to the high incidence of unbalanced karyotypes in their gametes. Preimplantation Genetic Diagnosis (PGD) can improve pregnancy and live birth rates by identifying only karyotypically balanced embryos for transfer. Currently, PGD of unbalanced translocations relies mainly on interphase fluorescence *in situ* hybridization (FISH) of blastomeres. Limitations of this technique include overlapping signals, dual signals due to replicated DNA, stochastic probe hybridization failures, and lost blastomeres, resulting in either misdiagnosis or uninterpretable data. We describe an alternative microsatellite PCR-based strategy for PGD of unbalanced chromosomal translocations. PGD was performed on a karyotypically normal woman and her husband, who carries a balanced translocation between chromosomes 12 and 22 [t(12;22)(p11.2;q11.2)]. Twenty oocytes were recovered after ovarian stimulation, 13 fertilized after intracytoplasmic sperm injection, and 8 developed sufficiently for blastomere biopsy. Blastomeres isolated from Day 3 embryos were subjected to a single-round multiplex-PCR amplification of microsatellite markers mapping to chromosome bands 12p13.2, 12q21.33, and 22q13.2. Three embryos were chromosomally balanced, four were unbalanced, and one showed discordant results in the two isolated blastomeres, suggestive of somatic mosaicism. Two chromosomally balanced embryos were transferred on day 4 and a singleton pregnancy, with 46 XY normal karyotype as confirmed by amniocentesis, ensued resulting in the birth of a healthy baby boy. This PCR-based strategy represents a viable alternative to FISH-based PGD testing of chromosomal translocations that may be preferred under certain situations.

P05.42 Neonatal screening for phenylketonuria in Kazakhstan

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Since 2006 year the national program of neonatal screening for phenylketonuria (PKU) and congenital hypothyroidism was introduced in Kazakhstan. In 2006-2009 years 446 348 newborns were studied for PKU. The middle scope of neonatal screening in Kazakhstan was 77%.

The program of neonatal screening revealed 19 newborns with PKU, that in further was conduct molecular-genetic researches in PAH gene.

The evaluation of genetic polymorphism, specters and rates of mutations in PAH gene in Kazakhstan showed that our population has a different from other world population by mutations specter of PAH gene. More of PKU patients were compound- heterozygous that had more accepted R408W mutation or R261Q, IVS10nt546, IVS12nt1 mutations or other unknown and undefined earlier mutations. The V245V polymorphism was revealed in 7 exon of PAH gene (frequency of mutation is 0,20), that didn't conduct to change of PAH protein. In health control group R408W and R261Q mutations PAH gene didn't reveal, that was evidence of low rate of heterozygous carriers these mutations in Kazakhstan population. Accounting ethnic structure of population frequency of PKU was 0,69 for 100 000 population by kazakh and 1,56 for 100 000 population by russian. Consequently, reveal ethnic feature of alleles of PAH gene distribution in Kazakhstan allowed to accounting population features conduct molecular-genetic diagnostic

for PKU, and introduce neonatal screening allow to opportunely reveal and diagnose diseases before clinic manifestation.

P05.43 Do parents in Ireland understand the Newborn Screening Test?

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Introduction: The National Newborn Screening Programme was introduced in Ireland in 1966. It currently screens for phenylketonuria, maple syrup urine disease, galactosemia, homocystinuria and congenital hypothyroidism. Almost all of the nearly 70,000 babies born annually in Ireland participate in the newborn screening programme, yet the impression is that parents in general have a limited understanding of the purpose of the test.

Aim: Our aim was to determine if Irish parents understand the newborn screening test.

Methods: A questionnaire assessing knowledge on the screening test was administered to 200 parents of children attending a paediatric hospital during October 2009. A qualitative grading system was established and applied to assess parental knowledge:

Grading of Parental Knowledge

| Grade | Knowledge |
|-------|--|
| 0 | Did not know if child was screened |
| 1 | No/inaccurate knowledge |
| 2 | Knowledge of metabolic/screening aspects of test |
| 3 | Knowledge of principles of screening & name a specific disease |
| 4 | Knowledge of principles of screening & name 3 diseases |
| 5 | Global understanding of screening & name all diseases |

Results Summary

n=200

151 mothers, 49 fathers, 1 grandmother

175 Irish, 25 non-Irish

Age Ranges

| Age | <12 months | 12-24 months | 2-3 years | 4-6 years | 7-11 years | 12-18 years |
|--------|------------|--------------|-----------|-----------|------------|-------------|
| Number | 24 | 24 | 30 | 44 | 44 | 34 |

Overall Grading of Parental Knowledge

| Grade | % | Number of Parents |
|-------|----|-------------------|
| 0 | 3 | 6 |
| 1 | 75 | 151 |
| 2 | 14 | 29 |
| 3 | 6 | 11 |
| 4 | 1 | 2 |
| 5 | <1 | 1 |

Conclusion: Despite the fact that almost every Irish parent allows their child to have the newborn screening test done there seems to be little knowledge about its significance. Does this reflect inadequate information prior to the test, the circumstances at the time of testing or trust in the health care system?

P05.44 Study of imprinted genes expression PHLDA2 and PEG10 in spontaneous abortions

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Genomic imprinting is defined as an epigenetic modification that inactivates one allele of a gene in a parent-of-origin-dependent manner. Current research on foetal control growth has focused on genes that display imprinted expression. Miscarriage in the general reproductive population is very frequent and other genetic causes beyond chromosomal abnormalities could be involved in spontaneous abortions (SA), such as variations of expression in imprinted genes particularly those

related to foetal or placental growth. *PHLDA2* a maternally expressed gene, and *PEG10* a paternally expressed gene, are known to play roles in foetal growth and placental development.

Quantitative Real Time PCR using TaqMan Gene Expression Assays was performed to evaluate the gene expressions patterns of *PHLDA2* and *PEG10* genes in 35 foetal/placental samples from SA. Two house-keeping genes *GAPDH* and *ACTB* were used as controls. The results were analysed by REST 2009 software showed that *PHLDA2* gene was upregulated in the first trimester and both genes were upregulated in the second trimester.

Data from literature has implicated *PHLDA2* as a gene potentially more active at early gestational ages. According to the "parental conflict" theory, maternal expressed genes restrict growth, whereas paternally expressed genes enhance growth. It would be expected that in intrauterine growth restriction cases, *PEG10* would be downregulated. The majority of our cases were idiopathic SA, so upregulation or downregulation of a gene according to the "parental conflict" is not predictable. As far as we know this is the first study presenting data from human imprinted gene expression in SA cases.

P05.45 Polymorphisms of renin-angiotensin system and the risk of placental insufficiency among women of the Republic of Tatarstan

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One of the main reasons of pregnancy and birth complications, which can lead to perinatal morbidity and mortality, is pathology of fetoplacental complex. The most wide-spread pathology is placental insufficiency (PI), accompanied by hypoxia and intrauterine growth restriction and complicates almost all pregnancy diseases. The main mechanism underlying PI involves endothelial dysfunction that leads to disbalance of the metabolism among fetal and maternal organisms.

We investigated the association between polymorphisms of renin-angiotensin system and the risk of PI among women of the Republic of Tatarstan. We compared 90 women with PI to 80 women with a normal pregnancy and evaluated them for the following genetic variants: A-20C, M235T and T174M polymorphisms of the angiotensinogen gene and G2350A polymorphism of the angiotensin-converting enzyme gene.

Genomic DNA was extracted from samples of whole blood in accordance with standard phenol-chloroform method. These polymorphisms were genotyped using RFLP-PCR analysis.

We also quantified concentrations of cell-free DNA from maternal plasma, newborn baby's plasma and amniotic liquid. Cell-free DNA was obtained from plasma using the Lytech DNA Kit and measured on spectrophotometer NanoDrop 1000. It is shown that amniotic liquid and maternal plasma can be used as an accessible biological marker to research cell-free DNA with the aim of PI diagnostic.

P05.46 Pre-implantation Genetic Diagnosis (PGD) for Genetic and Metabolic Disorders-The Saudi Experience

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Saudi Arabian culture is highly consanguineous, with the first cousin marriages accounting for 60-70% of all marriages. Given the difficulties in management of genetic disorders, reproductive option for families affected with genetic diseases in Saudi Arabia is often limited to PGD which is permissible under the law and religion whereas prenatal diagnosis with the intent of termination of pregnancy is neither widely practiced nor socially accepted, although it is accepted under certain conditions.

KFSH&RC has been offering PGD for monogenic and chromosomal disorders, since 2001. A total of 45 pregnancies initiated. Of which, 25 healthy babies were born, 13 pregnancies are ongoing and 7 pregnancies were either biochemical or ended up with abortion.

In all these families PGD was undertaken using fluorescent PCR (F-PCR) and/or nested PCR with sequencing on a single cell, or Multiple Displacement Modification (MDA) to amplify the whole genome from a single cell. A singleton pregnancy ensure after transfer of one heterozygous and one/or normal embryo and prenatal diagnosis by CVS confirmed a normal pregnancy. This is the first report of successful PGD in different genetic disorders in Saudi Arabia, and the Muslim world.

P05.47 Prenatal diagnosis of chromosomal abnormalities in Republic of Moldova

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High frequency of chromosomal abnormalities at pregnant women of risk group with severe obstetrical anamnesis, recurrent abortions, miscarriages, stopped pregnancies in evolutions, determine early applying of non-invasive and invasive techniques of prenatal diagnosis. Screening tests for diagnosis of chromosomal abnormalities executed in necessary time during pregnancy could prevent born of children with severe and major malformations and mental deficiencies. Prenatal diagnosis procedures as ultra-sonography (fetal nuchal translucency (NT)), biochemical screening (evaluation of maternal plasma level of alfa fetoproteine (AFP), beta-human chorionic gonadotropin (hCG) in maternal serum) and cytogenetic analyses, are necessary for complex investigation during medico-genetic counseling.

Research included a group of 1648 cytogenetically, biochemically and ecographically examined pregnant women in the period 2007-2009. In 1314 cases was applied amniocentesis. Karyotyping applied in high-risk pregnancies at 16-18 weeks represents the most informative diagnostic method for detecting Down syndrome and other chromosomal abnormalities. In 72% of examined cases were detected maternal age and markers for chromosomal anomalies, decreased levels of alpha fetoprotein. Abnormalities were detected in 9 -12 % of examined cases.

Conclusion: Non-invazive prenatal diagnosis using biochemical analyses (AFP) and ultra-sonographic markers (fetal nuchal translucency (NT) at 10 to 13(+6) weeks of gestation) during first trimester of pregnancy has a high sensitivity associated with a detection rate of 95% for a false positive rate of 5%.

P05.48 A de novo prenatal diagnosis of Leri-Weill dyschondrosteosis in a 45,XY,t(Y;21) foetus: an example of a cytogenetic-molecular integrated laboratory.

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We present a case of prenatal diagnosis of a 45,XY,t(Y;21)(p11;p11). ish der (Y;21)(p11;p11)(CEPY+, SRY+, D21Z1+) by karyotype in a amniotic fluid sample of a 32-year-old G1P0 woman at 21 weeks of gestation because of an hyperecogenic focus in the mitral valve. Parent's karyotypes were normal. Molecular studies of SALSA MLPA P018 allowed to characterize the deletion limits that encompassed the SHOX gene and the 3' SHOX region adjacent in the foetus sample showing a deletion from 9333-L10292 to 5648-L06218 probe (from Xptel at less 1200kb). MLPA analyses in both parents were normal. A further study with microsatellite analysis (DXYS10092, DXYS10093, DXYS10096; DXYS233, DXYS6814) in the family confirmed and delimited the deletion until 800-850pb in the foetus sample. According to these results, parents received a genetic counselling referred to a posible Leri-Weill dyschondrosteosis (LWD) in the fetus. LWD is characterised by a disproportionate short stature with predominantly mesomelic limb shortening and Madelung deformity of the arm. Females tend to be more affected than males. After genetic counselling, the couple elected to continue with the pregnancy. A ultrasonography evaluation at 26 weeks revealed an abnormal position of both wrists. A boy was born spontaneous at 39 gestation's week with 53 cm, 3700g and no apparently skeletal abnormalities. A clinical follow up is under way. We will have new clinical signs in a new reevaluation by a medical endocrinologist in a few days when he will be 6 months old.

P05.49 Cytogenetic findings in 700 consecutive amniocenteses

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The aim of the study was to evaluate the frequency and structure of prenatal detected chromosome abnormality. In this paper we report results of 700 fetal karyotypes performed by cultured amniocytes. The cytogenetic analysis with GTG banding of amniotic fluid cells revealed 17 aneuploidies (2,42%) and 23 structural abnormalities (2.9%). Each cases has received genetic counseling. We found trisomy 21 (11 cases) that includes omogen trisomy 21 (8 cases), mosaic trisomy 21 (3 cases) one of them with 2 cellular clones 47,XX+21/46,XX, another with 3 cellular clones 47,XY+21/48,YYY+21/46,XY and the other of chimera 47,XX+21/46,XY. Other autosomal trisomies that were found are the following: omogen trisomy 18 (1 case), mosaic trisomy 18 (1 case), omogen trisomy 13 (1 case) and mosaic trisomy 22 (1 case). Poliploidia was found in the form of the triploidy 69,XXX (2 cases). We found 2 Robertsonian translocations: 1 case maternal trop(13;22) and 1 case de novo trop(21;21). We found also balanced translocations: maternal t(7;10)(p22;p12.1) 2 cases in the same family and maternal t(17;20)(q12;q11) 1 case. Pericentric inversions were showed in 10 cases: maternal inv(10) 1 case, maternal inv(9) 3 cases, paternal inv(9) 5 cases and de novo inv(9) 1 case. Other structural abnormalities includes: duplication dup(10)(q22.2-ter) due to maternal balanced translocations (4;10)(q22.2;q22.2-ter) 1 case, ring chromosome r(6) 1 case, marker chromosome 2 cases. We found also 4 cases of deletions: del(7)(q21-ter) 1 case, del(1)(p31.2-ter) 1 case, del(1)(q32.1-ter) 1 cases, del(X)(q26-ter) 1 case. The karyotype using classical banding is an informative method able to detect chromosomal abnormalities.

P05.50 Prenatal screening in the 1st and 2nd trimesters of pregnancy.

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Objectives: A comparative analysis of the results of combined screening in the 1st and 2nd trimesters of pregnancy.

Materials and Methods: There were 304 women examined at 9-13 and 16-22 weeks of gestation by way of booth invasive and noninvasive methods. The protocol of examination included ultrasound scanning, detection of -HCG and PAPP-A serum markers in the 1st trimester, and of HCG, AFP, and estriol in the 2nd trimester, computer processing with Life Cycle in the 1st trimester and PRISCA in the 2nd one; invasive prenatal procedures.

Results: Dynamic assessment of 42 risk group patients (13.8%) in the 1st trimester revealed four cases (9.5%) with fetal pathology: Down s syndrome (2), Edward s (1) and Patau s (1) syndromes. There were no chromosomal pathology of the fetuses of 64(21.3%) risk group women found in the 2nd trimester. All karyotype changes were registered in the 1st trimester.

Conclusion: A high rate of false-positive findings testified of the fact that none of any noninvasive means of diagnosis of fetal chromosomal pathology had an absolute specificity.

Screening programmes should be applied preferably in the 1st trimester. It is not expedient to perform dynamic follow-up in low risk group. Noninvasive screening in the 2nd trimester must be carried out only in case of its absence in the 1st trimester. In high risk group invasive methods must be considered in the 1st trimesters of pregnancy. At present only invasive methods of diagnosis are important to make a decision of the tactics of pregnancy management.

P05.51 Sequential contingent screening and ultrasound - effective screening?

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Background: Prenatal screenings tests of pregnancy is targeted to select women having high risk of aneuploidy. Sequential contingent screening is based on I trimester screening results. According to combined risk three categories can be calculated and different follow up tactics can be used.

Methods: Maternal serum markers were measured I (fb-hCG, PAPP-A) and II trimester (AFP, hCG, uE3), in keeping with at 11-13 and 15-17 weeks of pregnancy. Gestation age, length of the fetus (CRL 45-84 mm), *nuchal translucency* (NT) - was determined during the first trimester by ultrasound investigation. Serum markers were tested using the Immulite 2000 automated chemiluminescent immunoassay method and Prisca risk calculation software for statistical risk calculations.

High, intermediate and low risk groups of pregnant women were selected according to I trimester combined test.

Results: Retrospectively data of 1000 women (February to July 2009) were analyzed using I and II trimester screening results. 915 were younger women (<37 years) and 85 were older women (>37 years). About 55% of tested women in the young age group had normal I trimester screening results (low risk), high and intermediate risk had all women in the elder age group. During this period fetuses with aneuploidy were detected in 6 of 1000 pregnancies: 4 aneuploidy in the young age group and 2 in the old age group.

Conclusion: The combination of sequential contingent screening with ultrasound of markers is flexible, cost effectiveness and implementability of early serum screening.

P05.52 Perspectives in prenatal genetic testing

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Prenatal testing has proved extremely valuable for the prevention of generic disorders. However, it has a number of disadvantages, such as invasive procedures and delayed results.

The FISH (fluorescence in situ hybridisation) method and the amplification of short tandem repeat sequences through the polymerase chain reaction (PCR) of a DNA fragment have both reduced the time of issuing the results from 2-3 weeks to 1-2 days.

A more recent possibility allows the results to be issued in several hours. Real-time multiplex PCR amplification and the quantitative amplification of the DNA fragment discriminate with high sensitivity the 3:2 or 2:1 ratio between the copies of homologous chromosomes in the case of numerical chromosome anomalies. However, the method used to collect fetal tissue samples is an invasive one.

The development of techniques to isolate fetal DNA from the circulating maternal blood may eliminate the invasive procedures. Placental free (extracellular) DNA can be isolated from maternal plasma and PCR-amplified. In the 11-17 weeks of pregnancy, about 1-5% of the free DNA in the maternal circulation comes from the fetus, while the rest comes from the mother. This method has 95-100% specificity, but can detect only fetal DNA sequences of paternal origin, because the maternal sequences interfere with the maternal DNA. The fetal DNA sequences in maternal circulation are shorter than the total circulating DNA.

Prenatal screening and diagnosis methods improve the health of the population.

P05.53 Informative value of biochemical screening tests for the detection of major numerical chromosomal abnormalities by means of QF-PCR.

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We report here the screening results of 1135 fetal samples (785 amniotic fluids and 350 chorionic villi) using Quantitative Fluorescent Poly-

merase Chain Reaction (QF-PCR), a rapid assay for the detection of major aneuploidies of 13, 18, 21, X and Y chromosomes.

These cases were selected according to the following indications for the invasive procedure: biochemical screening tests performed on maternal serum (bitest or tritest) and increased nuchal translucency.

Fifty-six women had positive biochemical screening: 36 bitest (dosage of hCG and PAPP-A) and 20 tritest (computer-based analysis of AFP, hCG, fE3 and maternal age). Our analysis revealed 2 trisomies 21 among patients with positive bitest (PPV 5.7%) and 3 trisomies 21 among patients with positive tritest (PPV 15%). These data highlight the low informative value of biochemical screenings for the detection of chromosomal abnormalities.

The analysis of samples with indication of increased translucency (measurement of the thickness of translucent area below the skin in the back of the fetal neck) detected 18 pathological samples with a PPV of 20.7%, showing less false positives compared to the biochemical screening.

All the results of QF-PCR were concordant with karyotype analysis. Our results indicate that the biochemical screening has a high number of false positives inducing a large number of women toward a useless invasive procedure. Before performing a biochemical screening every pregnant woman should be adequately informed about the high rate of false positives of the tests that need to be confirmed by an invasive procedure with a probable negative outcome.

P05.54 Prenatal detection of selected aneuploidies - comparison of a CE marked IVD commercial test kit and a home-made QF-PCR assay

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Since 2000 more than 5000 samples were analyzed in National Genetic Laboratory with QF-PCR technique. Our home-made assay includes 11 polymorphic STR markers (4 located on chromosome 21, 4 - on chromosome 18 and 3 - on chromosome 13) which are routinely used in our practice. As an alternative, recently we have successfully tested the commercial kit for the analysis of aneuploidy by Aneufast TM (Genomed Ltd). In this preliminary study we have evaluated and compared the level of informativeness of the commercial CE marked in vitro diagnostic test kit and our home-made assay. To determine the heterozygosity of the STRs in both assays we have included the results from two groups of 250 prenatal samples analysed by two combinations of markers. We found that Aneufast was technically easier to use and the informativeness is high. However, the results showed that the home-made combination of markers is more suitable for prenatal detection of selected aneuploidies in Bulgaria.

| Comparison of heterozygosity and informativeness of both assays | | | | |
|---|------------------------|---------|---------------|---------|
| | Heterozygosity levels: | | | |
| | Aneufast TM kit | | Home-made set | |
| Markers on: | lowest | highest | lowest | highest |
| Chromosome 21 | 68.9 % | 84.3 % | 71.2 % | 86.8 % |
| Chromosome 18 | 67.7 % | 86.0 % | 63.8 % | 91.2 % |
| Chromosome 13 | 78.2 % | 84.6 % | 76.6 % | 84.5 % |
| Informative results for more than 2 markers per chromosome: | | | | |
| Combination of markers on: | Aneufast TM kit | | Home-made set | |
| Chromosome 21 | 77.9 % | | 79.9 % | |
| Chromosome 18 | 74.8 % | | 77.1 % | |
| Chromosome 13 | 81.2 % | | 81.8 % | |

P05.55 Indications for invasive prenatal procedures and aneuploidies detected by QF-PCR analysis

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First- and second- trimester serum screening (SS) together with ultrasound 2D/3D are widespread non-invasive methods for antenatal screening for common autosomal aneuploidies. Pregnant women with calculated high SS risk or/and abnormal ultrasound findings are counseled to proceed to amniocentesis. The amniotic fluids routinely are analyzed by cytogenetic or/and DNA analysis. A total of 17 229 blood serums from pregnant women were investigated in 2009 for first- and

second-trimester SS. An increased risk for chromosomal aneuploidies was calculated for 1 224 (7%) of them. During the same period 1 289 women with different indications (including increased risk from SS, abnormal ultrasound markers and/or advanced maternal age) were referred to our department for prenatal detection of chromosomal aneuploidies by DNA analysis. Samples were investigated by QF-PCR assay on automatic sequencer. We have found pathological results for 46 samples (3.5%): 22 were found to be with trisomy 21, 11 with trisomy 18, 2 with trisomy 13, 3 with XXX, 4 with XXY, 2 with XO and 2 with triploidy. Positive SS was indication for amniocentesis in 23 (50%) of the pathological cases, abnormal ultrasound findings - in 14 (30%), advanced maternal age - in 7 (15%) and positive family history - in 2. Our 10-years experience shows that DNA analysis for chromosomal aneuploidies is appropriate when the indications for invasive prenatal diagnosis are positive maternal serum screening or/and abnormal ultrasound markers. When advanced maternal age or positive family history for chromosomal disorders is present DNA analysis should be combined with cytogenetic analysis

P05.56 The use of a novel Real-time PCR assay for rapid prenatal diagnosis of trisomy 21 syndrome

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Trisomy 21 is one of the common chromosomal abnormality with frequency of 1/700-1/1000 live birth. Complete trisomy 21 is seen in 95% of case. Current diagnosis methods are based on karyotype and FISH technique. But these methods have some limitation, such as availability of live fetal cells and the need of high technical expertise. Final results are achieved in 2-3 weeks. In this study, we have successfully used a novel real time PCR assay (TaqMan probe based) for rapid diagnosis of trisomy 21. DNA was extracted from 20 normal samples and 25 trisomy 21 samples using salting out method. A target gene (DSCAM) on chromosome 21 critical region and a reference gene (PMP22) on chromosome 17 were selected for gene dosage analysis. The primers and probes were designed according to ABI guideline using Primer Express® software. For each sample, the difference between the threshold cycle of DSCAM and PMP22 genes (ΔCt) were determined. Then, calibrated ΔCt value ($\Delta\Delta Ct$, sample ΔCt - normal control ΔCt) was calculated. The ratio ($2^{-\Delta\Delta Ct}$) of target gene to reference gene for normal individuals (1 ± 0.01) and for 21 trisomy cases (1.67 ± 0.16) clearly discriminated the two groups. The result of the real time PCR analysis was confirmed by the result of karyotyping. Therefore real time PCR is a reliable and accurate technique for rapid diagnosis of trisomy 21.

P05.57 Foetal presentation of five Rubinstein-Taybi syndrome cases with CREBBP alterations

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Rubinstein-Taybi syndrome (RSTS; OMIM 180849) is an autosomal dominant developmental disorder characterised by facial dysmorphism, broad thumbs and halluces associated with mental retardation. RSTS is caused by mutations in the closely related *CREBBP* (60-65%) and *EP300* genes (3%). About 10% of *CREBBP* alterations are deletions. RSTS is often diagnosed at birth or during early childhood.

We report on 5 cases of well-documented foetal RSTS. Two cases were examined after death *in utero* at 18 and 35 gestation weeks (GW) and

3 cases after ultrasound abnormalities and termination of pregnancy at 26, 33+4 and 35 GW. A large gallbladder was detected in 2 cases and an unusual severe prenatal microcephaly was seen in one case. Fetal autopsy showed that all foetuses had large thumbs and 4 had facial dysmorphism. A whole gene deletion was found in one case. Molecular analysis in the 4 remaining cases showed an amino acid deletion in exon 30, 2 splicing variants around exon 22, and one frameshift mutation in exon 25. In the latter case, we also identified a duplication of at least 50 kilobases extending into the 3'flanking region of the gene. Parent's DNA sequencing was in favour of *de novo* variants.

In conclusion, *CREBBP* alterations in foetal RSTS are similar to those found in typical RSTS diagnosed in childhood. This report will contribute to a better knowledge of particular features in foetal RSTS and to improvement of this clinical diagnosis in antenatal cases. It shows the interest of genotyping, allowing reassuring genetic counselling.

P05.58 Evaluation of 31 selective abortions after counseling

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In Iran, induced abortion is a criminal offence except to save the life of the mother or keep the family away from OSR and Haraj. OSR and Haraj is a condition was explained by the law as "any big problem and suffer for Family". The parliament now gave the list of diseases which pregnant women could have an abortion by order of forensic medicine.

Two to three percent of infants were born with congenital abnormality, which mostly now caused by genetic problem. However, investigation preformed after sonographic detection of central nervous system or skeletal anomalies had highest diagnostic yield. From 31 selective abortions in Yazd forensic medicine organization from 2006 till now, 11 cases had fetal severe abnormally and the rest because of mother safety. Seven of these 11 cases were diagnosed by sonography and 4 others by genetic test in amniocentesis. These results show prenatal screening is very important even only by sonography and it as one of the non-invasive prenatal diagnostic techniques should be used in every pregnancy.

P05.59 Neonatal Silver-Russell Syndrome With The Unique Combination of Maternal UPD 7 and Mosaic Trisomy 7

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Trisomy 7 mosaicism has been diagnosed at least eleven times during the prenatal period by amniocentesis. Among these cases, three were associated with minor phenotypic abnormalities that can be observed in Silver-Russel syndrome (SRS). Only one of these cases had maternal uniparental disomy of chromosome 7 (UPD7).

We present the second case of prenatal diagnosis of trisomy 7 mosaic fetus associated with UPD7 (10% of SRS). A 43-year-old woman underwent amniocentesis at 19 weeks' gestation because of advanced maternal age. Fourteen percent of the colonies (8 colonies out of 17) analyzed displayed trisomy 7 (47,XY,+7); whereas 86% of the colonies demonstrated a normal karyotype. Maternal UPD7 was not tested during prenatal period. Sonographic examination noted intra-uterine growth retardation at 23 weeks' gestation. The parents were counseled and they were informed about the risk of SRS and they nevertheless decided to continue with the pregnancy. The medical examination at birth noted dysmorphic facial features of SRS without body asymmetry, hypotonia and hypospadias. DNA analysis with polymorphic markers showed a maternal heterodisomy of chromosome 7. Chorionic villus tissue culture showed trisomy 7 in forty percent of analysed cells. The karyotype obtained from lymphocytes was normal (46,XY). Analysis of urinary cells showed a mosaic trisomy 7 (16% of cells). These findings suggest that mosaic trisomy 7 may have been overlooked in some cases of UPD7. In this regard the urinary cells represent one tissue that can be easily studied, looking for this mosaic trisomy.

P05.60 Molecular genetic and cytogenetic analysis of whole genome amplified rare single cells in a setting mimicking microchimerism

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Strategies for single cell analysis in cell based non-invasive prenatal diagnosis have to meet two purposes: first, the unambiguous identification of the respective cell and second, the analysis of the target of interest. Current strategies rely on various combinations of immunocytochemistry, immunofluorescence, and fluorescence *in situ* hybridization (FISH) or PCR for assignment of the status of candidate fetal cell. Despite recent methodical refinements these techniques are restricted to either fetal trisomic or male cells or few DNA loci specific for the fetal genome.

To overcome the limitations resulting from analysing the DNA of a single cell we implemented isothermal whole genome amplification (iWGA) on the single cell level for subsequently enabling both molecular and cytogenetic analysis from one and the same cell. An aliquot of cells of the colon carcinoma line HT-29 spiked into peripheral blood mononuclear cells was immunostained for cytokeratin, nuclear counterstaining was done using TO-PRO-3 for establishing a group of double-positive candidate fetal cells. Single candidate cells were microdissected and forwarded to low-volume on-chip iWGA. Aliquots of the iWGA products were forwarded to DNA fingerprint analysis for confirmation of the genomic identity, sequencing, and metaphase comparative genome hybridization (mCGH). DNA profiles, sequencing, and mCGH data prove our iWGA method to be compatible with analytical methods of a wide range of resolution.

P05.61 Genome wide 250k SNP array analysis for diagnosing pregnancies with ultrasound anomalies

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To explore the application of the Affymetrix 250k SNP array platform in prenatal diagnosis, we performed array analyses on DNA from 69 patients with prenatally identified anomalies. DNA was isolated from (un)cultured amniocytes or chorionic villi, fibroblasts or blood cells. Analyses were performed after TOP (N=35), IUFD (N=15), during pregnancy (N=1), or postnatally (N=18). Prenatal karyotypes were reported normal (N=54), balanced (N=1) or unbalanced (N=3) and in 11 cases culturing was not possible. Parental samples were available to study inheritance patterns.

Array analysis revealed de novo (mosaic) CNVs (N=14) or UPDs (N=2) in sixteen cases, interpreted as highly likely clinically relevant. In three of these, the abnormality had already been reported after routine cytogenetic analysis, in nine normal or balanced prenatal karyotypes had been reported and in four culturing of cells had not been possible. Aberrations inherited from a healthy parent and most likely not clinically relevant were noticed in five cases and CNVs with unclear clinical relevance in two cases.

In summary, in 9/55 (16%) cases with normal or balanced prenatal karyotypes and in 4/11 (36%) cases with unknown karyotypes, genome wide SNP array analysis enabled the detection of clinically relevant aberrations that otherwise would have remained undetected, whereas in two cases, the clinical relevance of an aberration remained unclear. Moreover, in 3/3 cases already identified aberrations could be characterized in further detail. We conclude that genome wide SNP array analysis is a powerful tool that might replace routine karyotyping in diagnosing babies with ultrasound anomalies.

P05.62 Molecular karyotyping using SNP-array analysis in 100 cases of prenatal diagnosis

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Karyotype analysis of cultured cells has been regarded as the gold standard for prenatal diagnosis for over 30 years. Since the first application of this technique to prenatal testing in the early 1970's, this procedure has proved to be highly reliable for identifying aneuploidies and structural rearrangements in foetal cells obtained invasively by ei-

ther amniocentesis or chorionic villus sampling (CVS). Although highly reliable to identify aneuploidies, as well as large chromosomal rearrangements, a significant limitation of this technique is the resolution that is > 5-10 Mb. Currently, prenatal diagnosis is reliant on cell culture and the reporting of results represents a time of great anxiety for parents during a pregnancy.

In our laboratory we have collected, previous informed consensus, 100 samples of amniotic fluid for the following indications of examination: advanced maternal age, positive screening test, ICSI, eco-evidenced markers / malformations.

We have used 20 ml of amniotic fluid, taken at 17 week of gestation, of which 15 ml were used for karyotype analysis and 5 ml were used for SNP-array analysis. The SNP-array analysis has been performed with HumanCytoSNP-12 BeadChip of Illumina. The data have been analysed with KaryoStudio software.

In our cohort of 100 samples, the Illumina SNP platform detected these imbalances: one duplication >1Mb, three duplication 1Mb, eleven deletions <500 Kb.

We compare the data obtained using SNP-array analysis with karyotype analysis.

P05.63 Carrier screening for spinal muscular atrophy in Taiwan on 107,611 pregnancies during 2005-2009

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Background Spinal muscular atrophy (SMA) is the most common neuromuscular autosomal recessive disorder. The American College of Medical Genetics has recently recommended routine carrier screening for SMA because of the high carrier frequency (1 in 25-50) as well as the severity of the genetic disease. Large studies are needed to determine the feasibility, benefits, and costs of such a program.

Methods A prospective large population-based cohort study of pregnancies was investigated in 25 counties of Taiwan during a 4.5-year period, January 2005 through June 2009. Two different validated platforms were used for parallel *SMN1/SMN2* gene quantification: denaturing high-performance liquid chromatography and multiplex ligation-dependent probe amplification.

Results We found 2,262 individuals with one copy of the *SMN1* genotype, recognized to be SMA carriers, among the 107,611 pregnancies screened. The carrier rate in our population was approximately 1 in 48 (2.10%). Of these individuals, 47 couples were at high risk for having offspring with SMA after testing of partners or spouses 2,038 who were also determined to be SMA carriers. Prenatal diagnoses were determined for 43 pregnancies (91.49%), of which 12 (27.91%) fetuses were diagnosed with SMA; the prevalence of SMA in our population was 1 in 8,968.

Conclusions The main benefit of this program of SMA carrier screening was to reduce the burden of birth of an affected child. Additionally, we determined carrier frequency and genetic risk to clinical research and providing carrier couples genetic services, knowledge as well as generic counseling for health care.

P05.64 Prenatal DNA testing of monogenic diseases with a late onset in Yakutia

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Spinocerebellar ataxia type 1 (SCA1) and myotonic dystrophy (MD), neurodegenerative and neuromuscular monogenic diseases with late onset, are widespread in the Yakut population (frequency 38.6:100 000 and 21.3:100 000 respectively). Genetic counseling of 62 pregnant women at risk for SCA1 or MD (43 with SCA1 and 19 with MD) was carried out in 2002-2009 years. During this period 25 procedures of prenatal DNA testing for SCA1 were carried out, in 13 cases results of DNA tests were negative and in 12 - were positive. 10 women with

positive prenatal DNA test for SCA1 expressed a desire to terminate a pregnancy, and 2 women refused to terminate a pregnancy with SCA1 mutation fetus. 10 procedures of prenatal testing for the MD have been carried out; in 7 cases the results of DNA tests were negative. Two women from three with positive result of DNA testing refused to terminate a pregnancy and children with congenital form of MD were born in both cases.

P05.65 Structural chromosomal aberrations in early spontaneous abortions

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About 15% to 20% of pregnancies end in spontaneous abortion, mostly in the first trimester. It is accepted that approximately 50% of early fetal loss results from chromosome abnormalities.

Cytogenetic examination was performed by the direct preparation method using chorionic villi. Among the 321 karyotyped abortions between 4 and 13 weeks of gestation, 53,6% chromosomally abnormal specimens were found and majority of chromosomal anomalies (90%) were numerical.

Structural aberrations of the chromosomes were responsible for 3,43% of early miscarriages and parental karyotyping is required. In 6 of 11 habitual abortions with structural changes a familial balanced rearrangement was established, that result in an increased recurrence risk in future pregnancies. The structural chromosomal abnormalities that we encountered were divided into reciprocal chromosomal translocations (4), Robertsonian translocations (4), deletions (2) and iso-chromosome (1). The subsequent pregnancy outcomes for the three women having a reciprocal translocation was normal karyotype after amniocentesis. The mean maternal age was 29,8 years and there was no positive correlation of advanced maternal age with structural chromosomal aberrations.

Structural chromosomal imbalances were also independent on gestational age, depending of specific chromosomes involved, genes and size of the contained segment.

Cytogenetic analysis of the first trimester spontaneous abortion is useful to identify the reason for the miscarriage and prenatal diagnosis should be offered to carriers of a balanced chromosomal rearrangement because it may predispose to offspring with an unbalanced karyotype.

P05.66** Adverse effects of trichothiodystrophy DNA repair and transcription gene abnormalities on human fetal and placental development

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Effects of abnormalities in DNA repair and transcription genes in human prenatal life have never been studied. Based on novel clinical observations, we conducted a genetic epidemiologic study to investigate gestational outcomes associated with abnormalities in TTD nucleotide excision repair (NER) and transcription genes, namely, *XPD*, *XPB*, *TTD-A* and *TTDN1*. We compared pregnancies resulting in TTD-affected offspring (N=24) with respect to abnormalities during their antenatal and neonatal periods to population reference values. We also analyzed the expression patterns of the four TTD genes in normal human tissues. Significantly higher incidence of several severe gestational complications was noted in TTD-affected pregnancies. Compared to reference values, TTD-affected pregnancies were significantly more likely to be complicated by hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome (RR=35.7, 95%CI:7.6-92.5, P=0.0002), elevated mid-trimester maternal serum human chorionic gonadotropin (hCG) levels (RR=14.3, 95%CI:7.0-16.6, P<0.0001), small for gestational age (SGA)<3rd percentile (RR=13.9, 95%CI:7.4-21.1, P<0.0001), preterm delivery (<32 weeks) (RR=12.0, 95%CI:4.9-21.6, P<0.0001), pre-eclampsia (RR=4.0, 95%CI:1.6-7.4, P=0.006), and decreased fetal movement (RR=3.3, 95%CI:1.6-5.2, P=0.0018). Abnormal placental development may explain the constellation of observed abnormalities. To test this hypothesis, we analyzed the expression patterns of the four TTD genes in normal human tissue. We found high

expression of TTD genes in human placenta, above the mean of their expression in all organs. Temporal analysis suggested that while *XPD*, *XPB* and *TTDN1* were consistently expressed from 14 to 40 weeks gestation, expression of *TTDA* was strongly negatively correlated ($r=-0.7, P<0.0001$) with gestational age. Our results indicate an important role for *XPD*, *XPB*, *TTDA* and *TTDN1* gene products during normal human placental and fetal development.

P05.67 A rare association of holoprosencephaly with triploidy in second trimester pregnancy-case report

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Triploidy is one of the most frequent chromosomal aberrations found in first trimester spontaneous abortions. Although majority of triploid conceptions are lost in first trimester, few will still survive into the second trimester and even rare in third trimester and leads to intrauterine or neonatal death. Increased maternal age is not a risk factor. More likely diandric fetuses survive to the second trimester.

We present a case of prenatal diagnosis of triploidy into the second trimester (20 weeks of gestation) through CVS. It is an adolescent mother, 17 years old and the referral reason for prenatal test was ultrasound anomalies, characterized by severe IUGR, holoprosencephaly, cardiac septal defects and kidneys chists. Also enlarged placenta was present allowing only CVS procedure. The karyotype found was 69,XXX.

It is a rare case when pregnancy survive into the second trimester and also holoprosencephaly is associate with triploidy.

The recurrence risk for future pregnancy is low, about 1%. The opportunity of a future prenatal test is not specifically indicated. It is recommended mother monitoring of hCG because of small risk of malign transformation.

P05.68 Application of quantitative Real-time PCR technique with SYBR Green I dye on amniocyte samples for prenatal diagnosis of trisomy 21

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Introduction: Trisomy 21 syndrome (Down syndrome) is the main causes of mental retardation and one of the most common chromosomal abnormalities (1 in 700-1000 births). Therefore diagnosis and prevention of live-born affected fetus is a health care priority.

Objective: Diagnostic Value of Real time PCR technique for prenatal diagnosis of trisomy 21 was evaluated.

Method and Material: Amniocentesis was performed on 59 pregnant women who had the high risk criteria of having fetus with trisomy 21. 15-20 milliliter of amniotic fluid was used for cytogenetic analysis and DNA extraction. 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 for these genes 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 C $\Delta\Delta$ t).

Results: The results of target/reference genes ratio in trisomy 21 and normal fetus was 1.61 ± 0.09 and 1.03 ± 0.05 , respectively that showed statistically significant difference between two groups.

Conclusion: Currently, the methods which are based on cytogenetic analysis are used to detect trisomy 21. These methods are generally time-consuming and also need cultured live cells. In contrast, Real-Time PCR is a high throughput molecular technique with none of the above mentioned limitations. Therefore, Real-Time PCR technique can be used as a rapid and reliable method for prenatal diagnosis of trisomy 21.

P05.69 Rapid Prenatal diagnosis of trisomy 21 syndrome using quantitative Real-Time PCR on Chorionic Villus Samples

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Trisomy 21 syndrome (Down syndrome) is an intricate neurodegenerative disease caused by a chromosomal aberration with the frequency

of about 1 in 800 births. Thus, prenatal diagnosis and termination of pregnancies with affected fetus is a health care priority. To this end, we have developed a novel quantitative Real-Time PCR method for prenatal diagnosis of Down syndrome. Chorionic Villus Samples (CVS) was obtained from 25 pregnant women who had the high risk of having fetus with Down syndrome in preliminary screening. Blood samples from 6 Down syndrome patients were used as positive controls. Genomic DNA was extracted using a commercial kit and quality and quantity of the DNA was measured by spectrophotometry. DYRK1A22, DSCAM (on chromosome 21) and PMP22 (on chromosome 17) genes were selected as targets and reference gene, respectively. Specific primers were designed and PCR efficiencies were determined for each gene. Gene dosage study was performed using quantitative Real-Time PCR and target /reference genes ratio was calculated according to the $2^{-\Delta\Delta Ct}$ formula. 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, respectively. The assay was able to demonstrate the presence of three copies of target genes in affected samples. DSCAM / PMP22 and DYRK1A22 / PMP22 ratio were significantly higher in trisomy 21 cases (1.5 times). Therefore quantitative Real-Time PCR technique can be used as a sensitive, accurate and reliable technique for rapid and prenatal diagnosis of trisomy 21 syndrome.

P05.70 Confined placental mosaicism of 22 trisomy with normal prenatal echosonography and normal eutrophic babyborn : trisomy rescue demonstrated by presence of fetal uniparental isodisomy.

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A chorionic villous sampling (CVS) performed at 11 weeks of gestation show at direct examination a trisomy 22 but after culture only a mosaicism (mos46,XX[15]/47,XX+22[5]) was detected. The US was strictly normal, without the classical IUGR leading to new investigations.

Cytogenetic analysis from amniotic fluid and fetal blood sample show normal 46,XX karyotype.

Molecular analysis show a maternal isodisomy (meiosis II) involving trisomy rescue, usual incrimined but rarely demonstrated.

At birth the baby was a clinically normal healthy little girl. As in earlier reports, Chromosom 22 seems not to show any apparent imprinting effect.

When there is clinical and biological discordance during pregnancy, Genetic counselling must be cautious and precise.

P06 Cancer genetics

P06.001 Correlation of p53, p16, p21 and MDM2 protein expression in human esophageal squamous cell carcinoma

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Backgrounds: Tumor suppressor genes p53, p21Waf1/Cip1 and p16INK4a and the proto-oncogene MDM2 are considered to be essential cell cycle regulatory proteins whose loss of function is associated with esophageal esquamous cell carcinogenesis [ESCC]. This study was conducted to investigate the immunohistochemical expression of these proteins in 80 ESCC patients in the northeastern Iran in which ESCC has the highest incidence of cancer, well above the world average.

Methods: Expression of cell cycle regulatory proteins in both the RB and p53 pathways was investigated Immunohistochemically, on tumor tissues of 80 ESCC patients and 60 available paraffin-embedded blocks of adjacent normal specimens of cases, along with normal esophageal tissues of 80 healthy subjects.

Result: Abnormal accumulation of the p53 protein along with p21 over-expression and p16 negative expression, but not MDM2, was significantly higher in the tumor tissue. ($P < 0.001$) Moreover, P53 positive

expression was observed in 10% of normal tissues adjacent to tumor, compared to none in the noncancerous control group.

Conclusion: These results suggest that disruption of the RB and p53 pathways may play a critical role in the development of ESCC among high-risk population of northeastern Iran. Moreover, detection of abnormally accumulated p53 in the morphologically normal tissue adjacent to tumor may be a predictor of future recurrence of tumor in these patients.

P06.002 Mutation analysis of the AATF gene in breast cancer families

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Background: About 5-10% of breast cancer is due to inherited disease predisposition. Currently known genes account for less than half of the hereditary cases. AATF (apoptosis antagonizing transcription factor alias CHE1) plays an important role in the DNA damage response and cell-cycle checkpoint control. Many previously identified susceptibility factors act in similar functional pathways as AATF, suggesting possible involvement of AATF in heritable breast cancer susceptibility.

Methods: We have screened affected index cases from 124 Finnish breast cancer families for germline defects in AATF by using conformation sensitive gel electrophoresis (CSGE). All alterations seen were characterized by direct sequencing with the Li-Cor IR² 4200-S DNA analysis system.

Results: Altogether seven different sequence changes were observed. To our knowledge, this is the first study reporting the mutation screening of the AATF gene in familial breast cancer.

Conclusions: The identification of novel susceptibility factors offers exciting challenges, because more than 70% of the genetic predisposition to breast cancer remains unexplained. The result of our current study indicates that germline alterations in the AATF gene do not significantly contribute to the risk of getting breast cancer.

P06.003 Translocation detection in Iranian patients with Acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is a fast-growing cancer of white blood cells. Lymphocytes are a type of WBC that the body uses to fight infections. In ALL, the bone marrow makes abnormal amounts of unformed cells called blasts that normally develop in lymphocytes. However, the blasts are abnormal. They do not develop and cannot fight infections. ALL is the most common cause of leukemia in children with an annual incidence of 3.5 cases per 100,000 children below 15 years of age. 60% of ALL is diagnosed in children below 15 years of age, and about 7% are diagnosed in young adults of 15-20 years of age.

In this study we analyzed leukemic patients by nested multiplex reverse transcription PCR (RT-PCR) for rapid diagnosis and screening, for 4 chromosomal translocation; t(1;19) (q23;p13) , t(12;21) (p13;q22), t(4;11) (q21;q23) and for t(9;22) (q34;q11).

Blood samples from the patients admitted to our genetic center were subjected to RNA isolation and cDNA synthesis. To achieve maximal sensitivity nested PCR protocol was used and a housekeeping gene was considered as an internal positive control.

We investigated 123 patients and the following results were obtained: 8 (6.5%) were positive for t(12;21) (p13;q22) , 6 (4.8 %) had t(4;11) (q21;q23) , 11 (8.94%) were positive for t(9;22) (q34;q11), which among them 3 (27.27%) had the 'b2a2', 5(45.45%) 'b3a2' and 3(27.27%) 'e1a2' breakpoint. Non of the patients had t(1;19) (q23;p13).

Our results were in concordance with other researches performed in different countries.

P06.004 The correlation pattern of acquired copy number changes in 164 ETV6/RUNX1-positive childhood acute lymphoblastic leukemias

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The *ETV6/RUNX1* fusion gene, present in 25% of B-lineage childhood acute lymphoblastic leukemia (ALL), is thought to represent an initiating event, which requires additional genetic changes for leukemia development. To identify additional genetic alterations, 24 *ETV6/RUNX1*-positive ALLs were analyzed using 500K single nucleotide polymorphism arrays. The results were combined with previously published data sets, allowing us to ascertain genomic copy number aberrations (CNAs) in 164 cases. In total, 45 recurrent CNAs were identified with an average number of 3.5 recurrent changes per case (range 0-13). Twenty-six percent of cases displayed a set of recurrent CNAs identical to that of other cases in the data set. The majority (74%), however, displayed a unique pattern of recurrent CNAs, indicating a large heterogeneity within this ALL subtype. As previously demonstrated, alterations targeting genes involved in B-cell development were common (present in 28% of cases). However, the combined analysis also identified alterations affecting nuclear hormone response (24%) to be a characteristic feature of *ETV6/RUNX1*-positive ALL. Studying the correlation pattern of the CNAs allowed us to highlight significant positive and negative correlations between specific aberrations. Furthermore, oncogenetic tree models identified *ETV6*, *CDKN2A/B*, *PAX5*, *del(6q)*, and *+16* as possible early events in the leukemogenic process.

P06.005 *NPM1*, *FLT3* and *c-KIT* genes mutations in childhood AML in Russia

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Acute myeloid leukemia (AML) is a genetically heterogeneous clonal disorder characterized by the accumulation of acquired somatic genetic alterations in hematopoietic progenitor cells. Approximately 50% of patients with AML exhibit a normal karyotype. Somatic mutations affecting transcription and signal transduction were identified in cytogenetically normal AML in the nucleophosmin 1 (*NPM1*), the fms-related tyrosine kinase 3 (*FLT3*), and the tyrosine kinase *c-KIT* genes, which makes the mutations important prognostic markers. In present study we investigated the frequency of mutations in childhood AML in Russian population. Genomic DNA was extracted from 130 samples from patients with AML. *FLT3* gene mutations of the internal tandem repetition ITD and activation loop *FLT3/D835Mt*, *NPM1* gene mutations of exon 12 and *c-KIT* gene mutations of exons 8 and 17 were examined by PCR and direct sequencing. In parallel, the samples were analyzed for the presence of translocations using diagnostic biochip.

We have found *NPM1* mutations in 7 of 130 patients (type A tcgt - 3, type B catg - 2, type D cctg - 1 and type Qm tcgg - 1). The *FLT3*/ITD mutation appeared in 9 of 130 patients, the length varied from 11 to 110bp, whereas other *FLT3* and *c-KIT* mutations were not found. The rates of mutations are significantly lower in childhood AML as compared with adult AML (published for Japanese and Italian populations): *NPM1* gene mutations - 5.4% (7/130) against 25-52%, *FLT3*/ITD - 6.9% (9/130) against 18.9%. The work was supported by the Russian Foundation for Basic Research (project no. 08-04-01480).

P06.006 Frequency of mutations in *NPM1* and *FLT3* genes and their impact on prognosis in patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) in Russia.

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Mutations of the nucleophosmin (*NPM1*) and Fms-like tyrosine kinase 3 (*FLT3*) genes have recently been described as the most frequent mutations in acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). Frequencies of these mutations and their impact on clinical course have been determined around world, but not in Russian population. Our aim was to investigate frequencies and prognostic

relevance of *NPM1* and *FLT3* mutations in patients with AML and MDS in Russia. We have studied blood samples from 44 patients with secondary and de novo MDS and 43 patients with AML. It was shown that most of the patients with de novo MDS carried *NPM1* mutations in contrast to the patients with secondary MDS, and both groups had low frequency of *FLT3* mutations. However *FLT3* mutations were found to be predominant in patients with AML. It was shown that total frequency of *FLT3*-ITD, *FLT3*-TKD and *NPM1* 4 bp insert mutations was 37.2% of all studied AML cases where *FLT3*-ITD mutation was the major (13.9%). Taken together our results allow us to conclude that mutation in *NPM1* gene in MDS patients with normal karyotype leads to favorable prognosis for disease outcome; *FLT3*-ITD mutation in AML *NPM1*- patients with normal karyotype leads to poor prognosis.

P06.007 Identification of regulatory genetic variation in childhood acute lymphoblastic leukemia (ALL)

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Acute lymphoblastic leukemia (ALL) is the most common form of childhood cancer. Although treatments for ALL currently exist, some patients do not benefit from them, while others suffer from unnecessary side effects. In order to gain insight into regulatory genetic variation that affects drug response in ALL, we have previously measured the allele-specific expression (ASE) levels of over 8000 genes in RNA-samples from 197 ALL patients whose *in vitro* drug responses against ten anti-cancer drugs are known (Milani et al. Genome Research 2009). Guided by the allele-specific differences in gene expression, we chose a subset of 56 genes displaying ASE for re-sequencing. We are sequencing the whole genes, including exons, introns, and some of the 5' and 3' non-coding sequence making up a target region of 3.1 megabases in total, in order to find the regulatory variants. We have used Nimblegen sequence capture arrays and Agilent's Sure-Select method for preparing sequencing libraries from DNA samples from ALL patients. Using the Illumina Genome Analyzer, we have sequenced libraries prepared from 63 ALL samples. We have used BWA for sequence alignment and the Samtools package for further analysis of the sequencing data and called up to 3500 SNPs per sample. We are now selecting the regulatory variants discovered by sequencing for genotyping in a larger set of patient samples. We will correlate the genotypes with the allele-specific expression levels as well as with the drug response patterns of the patient cells.

P06.008 Androgen receptor CAG polymorphism and breast cancer risk in Macedonian women

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Androgens have been hypothesized to influence risk of breast cancer through several mechanisms, including their conversion to estradiol or their binding to the estrogen receptor and/or androgen receptor (AR) in the breast. Androgenic stimulation may oppose breast cell proliferation, that it is mediated by AR. The first exon of AR gene contains a translated polymorphic CAG (poly-glutamine) repeat (from 8 to 35 in normal population), and its' length is inversely correlated to the trans-activation power of the receptor. Longer CAG repeats have been associated with increased risk of breast cancer. The aim of this study was to examine whether AR-CAG repeat lengths are related to breast cancer susceptibility in Macedonian patients with breast cancer. We studied 71 patients with breast cancer and 76 controls. The CAG repeat number was determined by fluorescent polymerase chain reaction (PCR) amplification of exon 1 of the AR gene and capillary electrophoresis on ABI3130 Genetic Analyzer. Comparisons were made for mean allele length, and separately for the shorter and the longer alleles. Dichotomous categories for CAG repeats were generated at all possible cut-points. The AR CAG repeat ranged from 10 to 34 in all tested women. The mean number of the repeats was not statistically different between patients (22.32 \pm 1.96) and controls (21.81 \pm 1.97). We found a significantly higher percentage of longer alleles \geq 25 repeats in breast cancer patients than in controls ($p=0.0002$, O.R. (1.87-8.32).

This finding suggests that women with longer allele ≥ 25 CAG repeats may be at increased risk of breast cancer.

P06.009 Antimutagenicity and Anticancer effects of *Citrus Limon*

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Background: Currently cancer is considered as one of the main factors of mortality globally. Many chemicals in our environment can cause genetic mutations and are potentially responsible for millions of cancer-related deaths. The scientist are nowadays looking for food materials which can potentially prevent the cancer occurrence. The purpose of this research is to examine antimutagenicity and anticancer effect of *Citrus Limon* fruit juices.

Material and methods: In present study Human Astrocytoma cancer cells were cultured in DMEM (Gibco), supplemented with 10% fetal calf serum, penicilin-streptomycin, L-glutamine and incubated at 37 °C for 2 days. In addition cancer cell line were treated by *Citrus Limon* fruit juice and Cellular vital capacity was determined by MTT. The *Citrus Limon* fruit juice was subsequently evaluated in terms of antimutagenicity and anticancer properties by a standard reverse mutation assay (Ames Test).

Results: During MTT, human Astrocytoma cell line revealed to have a meaningful cell death when compared with controls ($p < 0.01$). In Ames Test the fruit juices prevented the reverted mutations and the hindrance percent of half-ripe *Citrus Limon* was 71.7% and ripe *Citrus Limon* was 34.4% in Antimutagenicity test and this value in anticancer test was 83.3% and 50% in half-ripe *Citrus Limon* and ripe *Citrus Limon* respectively.

Conclusion: This is the first study that have revealed antimutagenicity and anticancer effect of *Citrus Limon* fruit juice and the effects were higher in half-ripe *Citrus Limon* in comparison to the ripened one.

P06.010 Splicing mutations of the APC gene in the Czech FAP families

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Familial adenomatous polyposis (FAP) is an autosomal dominant syndrome with almost 100% risk of colorectal cancer. The typical FAP is characterized by hundreds to thousands of colorectal adenomatous polyps and by extracolonic manifestations. An attenuated form of FAP (AFAP) is characterized by less than 100 adenomas and later onset of the disease.

We analyzed the APC gene for germline mutations in 386 FAP/AFAP patients. Mutation screening was performed using DGGE. DNA fragments showing an aberrant electrophoretic banding pattern were sequenced. All APC-mutation-negative probands were screened for large deletions of the APC gene using multiplex ligation dependent probe amplification (MLPA). Analysis of mRNA variants followed in probands with possible splicing mutation. This analysis was performed by PCR amplification of target site flanking exons and sequencing the normal and aberrant products.

We identified 88 germline mutations among 144 unrelated probands including large deletions. 11 germline APC mutations detected last two years have not been reported yet, which gives evidence of great variability of mutations.

At all, 15 of the mutations would possibly cause splicing errors. Analysis of mRNA of nine of these patients showed exon skipping in 6 cases, change of the amount of alternatively spliced product in one case, and no effect in two cases. Several previously unknown alternative splicings of APC gene were also revealed in normal controls.

This research was supported by the VZ MSM0021620808 of the Czech Republic.

P06.011 Study of cloned gene-related protein Vp2 of Infectious Bursal Disease Virus- induced apoptosis in human B cell lymphoma *in vitro*

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Background: Apoptosis or programmed cell death (PCD) is an important mechanism in both development and homeostasis in adult tissue for the removal of superfluous cells that its inducing is an effective cure of cancer. The aim of the present study were to evaluated the effect of cloned gene-related protein Vp2 of Infectious Bursal Disease Virus in human lymphoma B cells.

Material and methods: In present study after cloning the Vp2 gene in *Pichia pastoris* system, the Vp2 protein was expressed. Cellular vital capacity was determined by MTT. Then effect of Vp2 protein in human lymphoma B cells was examined by Hoechst staining and Flowcytometry techniques, respectively.

Results: During MTT, human lymphoma B cell lines revealed to have a meaningful apoptosis at 1 μ g and 5 μ g protein concentrations when compared with controls ($p < 0.01$). Apoptotic bodies appeared by Hoechst staining apoptosis was induced suitably after 48 hours by Flowcytometry assay.

Conclusion: The present study is the first study that have revealed the gene-related protein Vp2 induced apoptosis in human lymphoma B cells *in vitro*.

P06.012 Genetic diagnostics of atypical teratoid/rhabdoid tumor in children in Slovakia

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Introduction:

Atypical teratoid rhabdoid tumor (AT/RT) is an aggressive type of embryonal tumor - WHO Grade IV. Most frequently it occurs in children under age 2. Due to its morphological variability, AT/RT is often falsely misdiagnosed for a primitive neuroectodermal tumor (PNET) or medulloblastoma. For the majority of AT/RT cases is characteristic either deletion, or chromosome 22 monosomy - loss of the *hSNF5/INI1* gene, which is localized on 22q11.2.

Methodology and results:

We examined paraffin embedded tumor tissue samples by a method of fluorescent *in situ* hybridization in three child patients diagnosed in 2008-2009. In order to assess diagnosis we used the IGL probe (22q11), by way of which we indirectly confirmed gene *INI1* deletion. Gene *INI1* deletion was directly validated by the proposed BAC probe RP 11-8007 (22q11.23).

A 2-year-old patient was diagnosed with AT/RT CNS at the age of 1, and one year later, a renal rhabdoid tumor was diagnosed in the very same patient. We assumed the presence of gene *hSNF5/INI1* constitutional inactivation (rhabdoid tumor predisposition syndrome).

Only scarcely a literature reference is found on metachronal renal rhabdoid tumor formation following an AT/RT of the brain.

Examining the patient's peripheral blood by the sequential method of molecular genetics revealed mutation of *c.395_396insCC p.Val132ValfsX12* in exon 4 of the *hSNF5/INI1* gene, which so far has not been described in NCBI, nor HGMD database. Further peripheral blood examination in patient's parents and sibling revealed no germline mutation in exon 4 of the *hSNF5/INI1* gene that was being monitored.

P06.013 Optimisation and standardisation of sample preparation with the Bead-beating technology in genomics research

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In the context of sample preparation and cell lysis, Bertin Technologies (France) has developed a technology dedicated to the homogenization and grinding of soft to hard materials. The goal is to improve the first critical step in any molecular biology process and follow the latest requirements of analysis equipments which have radically improved in terms of throughput, reproducibility, detection limits and linearity.

Following specific mechanical engineering studies of bead beating technology, a high speed figure-8 multidirectional motion gives shaking energy to the beads that grind/homogenize samples in sealed vials (2mL or 7mL). This patented solution Precellys24 plays a large part in the analyse chain of rapid method to extract full length DNA, stable RNA, native state proteins or drug compounds out any samples. Thanks to Cryolys option, temperature inside Precellys24 tubes is maintained at an optimal level during homogenization.

Bertin and its partners have been investigating mechanical lysis with the Precellys bead beater vs. manual, chemical or sonicator methods. Several applications on DNA and RNA extraction from human or animal tissues illustrate the contribution of this equipment to the improvement of genomics research.

Bead beating technology was successfully evaluated in these applications and satisfied users in term of efficiency without degradation of the material, reproducibility, time and labour saving that are mains items to consider.

P06.014 Molecular evidence supporting field effect in recurrent and primary multiple superficial bladder cancers

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High recurrence rate is a common phenomenon in superficial bladder cancer (SBC). Recurrences, as well as primary multifocality, might be explained with monoclonal theory, suggesting that all coexisting/following tumors arise from a single malignant transformed cell, which proliferates and spreads throughout the urothelium or might be spread during the intravesical manipulations. Another theory explains multifocality/recurrences as subsequent events secondary to a field-cancerization effect, so tumors are expected to be genetically non-identical. The question weather recurrent/coexisting tumors arise from the same tumor clone or develop independently has a great clinical relevance to surgery and treatment approaches. Molecular analysis of alterations patterns in each of coexisting/recurrent tumors may give us an answer.

We examined 86 tumor samples from 19 patients with recurrent SBC (RSBC) (primary tumor and 1-4 relapses) and 8 patients with primary multiple SBC (MSBC) (2-5 tumors/patient). Genomic DNA samples were prepared from formalin-fixed, paraffin-embedded sections. Our panel included LOH analysis at 9p21 and 17p13 (microsatellite assay), promoter hypermethylation of *RASSF1A*, *P16*, *DAPK* (methyl-sensitive PCR).

Fifteen of 19 (79%) patients with RSBC showed different molecular alterations pattern between primary and recurrent tumors as well as 7 of 8 (88%) patients with MSBC. Moreover, relapses from the same patient also had different alteration patterns. Four of 19 (21%) patients with RSBC and one of 8 (12%) patients with MSBC demonstrated concordant alteration pattern between primary and subsequent/coexisting tumors.

Our results show that the relapses and coexisting multiple tumors in many cases of SBC arise from independently transformed urothelial cells.

P06.015 Testing the antitumor effect of hemocyanins on genomics and proteomics level

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Urinary bladder cancer is the 7-th most common cancer worldwide and is a socially significant healthcare problem. Risk factors such as DNA mutation and environmental factors: tobacco smoking, infection by *Schistosoma haematobium*, diet and the aging of the world population may be responsible for its pathogenesis. One of the approaches for bladder cancer therapy is the use of Keyhole limpet hemocyanins (KLH). The hemocyanins are copper-containing respiratory proteins and serve as oxygen-carriers in the blood of some arthropods and

mollusks. Our laboratory investigates hemocyanins and their derivatives from the species: *Helix lucorum* (HIH) and *Rapana venosa* (RvH) and their clinical use. The aim of our study is to examine their effect on genetic level against bladder cancer.

Three different hemocyanin isopolypeptides, named β -HIH, α D-HIH and α N-HIH, were isolated from the hemolymph of the *Helix lucorum* and were identified by their N-terminal sequences and molecular masses. The molecular masses were determined by PAGE and size exclusion chromatography. The mass of the β -HIH was found to be 1068 kDa and the masses of α D-HIH and α N-Hc - 1079 kDa.

The anti-tumour effects were investigated on 647-V, T-24 and CAL-29 bladder tumor Gene expression profiling and Microarray CGH analyses of the tumor cells before and after haemocyanins treatment, and studies on the gene sequences of the haemocyanins isoforms will be discussed.

P06.016 STUDY OF GENE EXPRESSION IN SUPERFICIAL TRANSITIONAL CELL CARCINOMAS

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Treatment of bladder superficial tumours is dependent on the risk of recurrence and it is therefore clinically important to identify bladder cancers with a high risk of intravesical recurrence after transurethral bladder tumour resection. To improve the accuracy of tumour progression prediction various molecular markers have been evaluated by gene expression microarrays but to date no relevant molecular markers have been used in clinical practice.

For the improvement of recurrence prognosis we have applied gene expression microarray analysis to two groups of bladder tumours (superficial bladder tumours with no or late recurrence during the period of two years versus early recurrence ones).

Data from microarrays containing 29,019 targets (Applied Biosystems) were subjected to a panel of statistical analyses to identify bladder cancer recurrence-associated gene signatures. Initial screening using the GeneSpring and Bioconductor software tools revealed a putative set of about 50 genes differing in gene expression in both groups. After validation significant differences were observed by *ARHGEF4*, *NINJ1*, *PRICKLE1*, *PSAT1*, *TM4SF1* and *TNFSF15* genes. Besides, we integrated the microarray dataset with additional background knowledge, in order to algorithmically mine for differential-expression patterns in terms of the Gene Ontology functions and processes as well as known regulatory pathway memberships.

Our results indicate that it may be possible to identify patients with a high risk of disease recurrence at an early stage using a molecular profile present already in the superficial tumours.

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P06.017 RT-PCR Analysis of putative BRCA1/2 Splice Variants

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Introduction: A subset of the unclassified variants (UVs) found in the *BRCA* genes may affect splicing. Twelve putative *BRCA1/2* splice variants prior selected upon *in silico* analysis were studied experimentally using RT-PCR.

Methods: The variants selected were: in *BRCA1* c.693G>A and in *BRCA2* c.68-7T>A, c.425G>T, c.794-11T>C, c.6935A>T, c.6842-3T>C, c.6943A>G, c.7976+3del2, c.8350C>T, c.8953+13A>G, c.8662C>T and c.8754+3G>C. We performed short-term lymphocyte cultures, in the absence or presence of puromycin, a nonsense mediated mRNA-decay inhibitor. PCRs using primers flanking the region of interest were performed to look for aberrant cDNA. This was followed by allele-specific PCRs in order to analyze the contribution of each allele to the

relative expression of the wild-type and aberrant transcripts found. Results: The variants *BRCA2* c.425G>T, c.7976+3del2, and c.8754+3G>C result in aberrant splicing, i.e. exon 4, exon 17 skipping and retention of 46bp of intron 22, respectively. The *BRCA1* variant c.693G>A results in exon 11 skipping, which is a normal isoform in breast- and ovarian tissue but not in lymphocytes. *BRCA2* variants c.68-7T>A and c.6935A>T induce higher expression of the *BRCA2Δ3* and *BRCA2Δ12* isoforms, which are also expressed in controls, whereas the level of expression of the full-length transcript is the same as from the WT allele. For the other variants no splice aberrations were detected.

Conclusion: The *BRCA2* variants c.425G>T, c.7976+3del2, and c.8754+3G>C could be classified as pathogenic. The clinical relevance of variants that induce higher expression of normal isoforms is difficult to evaluate as their function is currently unknown.

P06.018 Two deleterious *BRCA1* and *BRCA2* mutations in a Spanish family.

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Double heterozygote (DH) families for *BRCA1* and *BRCA2* mutations are a rare event mostly found in populations with founder mutations. To date nearly 29 families have been described worldwide; most of them harbouring at least one of the three Ashkenazi mutations, whilst only three families are not associated with known founder mutations. Here we describe the first DH Spanish family without founder mutations. The index case was a woman with breast cancer (onset age 48 years) that carried the nonsense *BRCA1*-c.153C>T (p.Q12X) and the novel *BRCA2*-1815delTinsCA mutation. Other tumours observed in this family were ovarian, colorectal and three gastric cancer cases. Intriguingly, the daughter with ovarian cancer at 34 years only inherited the *BRCA1* mutation. Other unaffected relatives were a 30 years-old son that carried both mutations and a 46 aged nephew that inherited only the *BRCA2* mutation. Breast cancer lifetime risks of 12.8% and 6%, respectively, were previously established with BRCAPRO, thus, the former patient was included in the surveillance program. This is the only double heterozygote found in 860 families scanned for BRCA mutations until now (0.12 %), a frequency similar to those reported in other European countries.

Whereas *BRCA1*-c.153C>T mutation has 13 records in BIC mutation database, *BRCA2*-1815delTinsCA is a new mutation present in two more unrelated families of our series. Haplotype analysis of twelve polymorphic markers linked to *BRCA2* suggested a common origin for this mutation.

P06.019 *BRCA1* and *BRCA2* mutation in Iranian breast cancer patients

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Introduction: *BRCA1* mutations are responsible for a significant proportion of hereditary breast and ovarian cancer (HBOC) families. *BRCA1* is responsible for more than 50 % of HBOC families with at least four cancer cases. Penetrance may be modified by other risk or protective genes or environmental factors. Mutations in *BRCA1* may play an important role in evaluation of sick risk, earlier diagnosis and gene therapy of breast cancer in Iranian populations

We conducted a study investigating *BRCA1/2* among 60 Iranian breast cancer patients with a personal or family history suggestive of hereditary predisposition to breast cancer. Total genomic DNA was extracted from 60 idiopathic breast cancer Patients and 40 cases of healthy people. Primers were designed to amplify the 20 hot exons. The entire *BRCA1* coding sequence was amplified by PCR with primers especially designed for comprehensive mutation screening by single-strand conformation polymorphism (SSCP) analysis. analysis and alterations were confirmed by DNA sequencing

The distribution of the breast cancer mutation in the control samples matched the distribution reported for control samples by others. The analysis of our sample shows no difference between the analysis of exon 11gene from 60 DNA samples of breast cancer patients showed no mutation. Our results suggest that (1) *BRCA1/2* mutations are seen in low -risk Iranian women with breast cancer. However this does not

exclude the association of the gene in breast cancer but it needs more investigation. Also it is necessary to test the other exons of the gene

P06.020 The comprehensive molecular-genetic analysis of hereditary breast and ovarian cancer syndrome: *BRCA1* and *BRCA2* genes

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Germline mutations in the *BRCA1* and *BRCA2* genes account for the majority of hereditary breast ovarian cancer (HBOC) cases. The analysis of *BRCA1* and *BRCA2* genes in 376 Slovak HBOC families revealed in 70 families a presence of relevant mutations, what represents 18.6%. We observed that the best clinical criterion for *BRCA1* analysis is familiar occurrence of disease and diagnosis of breast cancer at the age around 40 years together with the presence of ovarian cancer diagnosed at the age around 50 years in the family. In *BRCA2* analysis it is an exclusive presence of breast cancer without any ovarian cancer diagnosed at the age over 45 years in the family. We have identified three novel, probably Slovak specific pathogenic mutations c.80+3del4 and c.1166delG in *BRCA1* gene and c.6589delA in *BRCA2* gene and also the presence of a very rare large genomic rearrangement affecting complete *BRCA1* allele, deletion of exons 1 to 24 respectively.

Increasing the effectiveness of the analysis may be enriched by enlargement of analysed sequence of *BRCA* genes and by more stringent selection of HBOC families directed for *BRCA1/2* analysis.

P06.021 Acceptability of breast cancer medical prevention by letrozole in post- menopausal women with a *BRCA1/2* mutation in the LIBER trial

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Women carrying a germline *BRCA1/2* mutation have a life-time risk of developing breast cancer of 56 to 80%. Prophylactic bilateral mastectomy provides a valid option to reduce breast cancer incidence, but greatly affects the quality of life. There is therefore an urgent need to evaluate medical preventive alternative.

The major breast cancer prevention trials using tamoxifen showed an approximately 50% incidence reduction in high risk women. Adjuvant trials comparing aromatase inhibitors (AI) to tamoxifen revealed a higher preventive efficacy of AI for contralateral cancer with fewer thromboembolic side effects.

The French federation of cancer centres („FNCLCC“) has developed a randomized phase III study to determine the efficacy of letrozole to prevent breast cancer in postmenopausal *BRCA1/2* carriers. The „LIBER“ study is a double-blinded, letrozole versus placebo study involving 32 centres. The study opened for recruitment in march 2008. Here we present data reflecting the acceptability of this preventive trial. Twenty-one centres replied to an inquiry. 690 women were eligible. Out of 485 women informed by letter, 217 (44%) came to consultation and 62 (13 %) entered the study. The main concerns of women while considering to enter the trial were: the potential side effects, the probability to receive the placebo and the lack of support from other practitioners. For post-menopausal women bearing a *BRCA1/2* genetic predisposition, prevention of breast cancer risk by letrozole could provide a precious alternative to bilateral mastectomy. The acceptability of this phase III randomized double-blinded letrozole versus placebo trial by patients who received oral information is 28%.

P06.022 The large *BRCA1* new deletion revelation among Russian families with breast/ovarian cancer.

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Inherited predisposition to breast/ovarian cancer is mainly due to BRCA1/2 gene mutations. The methods for point mutation determination cannot detect large deletions or insertions that include one or more exons. Meanwhile, a frequency of such gene rearrangements may have significant portion from a frequency of point mutation and may be different among populations. Large rearrangements are more frequent in BRCA1 gene. In present work large rearrangement finding was conducted among families with inherited breast/ovarian cancer by analysis of SNP haplotypes, following gene alteration studing by RT-PCR and sequencing in the deletion vicinity. Among 58 heterozygous on BRCA1 haplotype B samples one deletion was revealed (1.7%). The deletion is unique and was not found earlier in the other populations. The deletion encompasses exons 14-17 and has a size 11327 bp. The 5' breakage point is disposed in AluSp that is in composition of four Alu tandem. In this tandem disposed also the breakage point of BRCA1 exons 14-20 known deletion. The 3' breakage point is disposed in AluSg near of which (100 b.p.) is Alu containing the breakage point of BRCA1 exons 17 known deletion. It is possible that these regions are hot-spot of recombination.

P06.023 Identification of a unique de novo BRCA1 mutation in a patient diagnosed with breast and ovarian cancer in her fifties

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Germline BRCA1&2 mutations confer high risks for breast and ovarian cancer and are most prevalent in patients with a family history for the disease. De novo BRCA mutations are rare and more frequently reported in BRCA2 (6 cases) than in BRCA1 (1 case), all in patients with tumors occurring before the age of 40. The case presented below illustrates that de novo mutations do not only occur in patients with early onset disease.

We analysed the complete coding region of BRCA1&2 in 169 sporadic breast and/or ovarian cancer patients with early onset or bilateral or multifocal tumors. In 16 (9.5%) a germline BRCA1/2 mutation was identified; in three patients these were potentially de novo, unfortunately, parental DNA was not available for 2 of them. Here, we report a patient (diagnosed with breast cancer at 52 yrs and ovarian cancer at 53 yrs) and heterozygous for a novel BRCA1 mutation c.3494_3495delTT (p.Phe1165fs). This mutation was absent in her parents (paternity confirmed) and 8 sibs as verified by Sanger sequencing, but was transmitted to 2 of her sons. To investigate possible mosaicism, the relevant amplicons were deep sequenced with 454 amplicon sequencing. The mutation in the patient was detected in 43% of the reads at 157 times coverage, but was absent in the maternal and paternal sample both covered 1840 and 2780 times respectively, consistent with the de novo occurrence (although germline mosaicism cannot be ruled out). Studies to determine if the mutation originated on the maternal or paternal allele are ongoing.

P06.024 Prevalence of BRCA1 mutation in Greek high-risk ovarian cancer patients

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Germline mutations in the BRCA1 and BRCA2 genes contribute to the majority of hereditary ovarian cancers and comprise 10-18% of total cases. The lifetime risk for ovarian cancer in BRCA1/2 mutation carriers ranges from 15% to 40%. A variable incidence of mutations has been reported for these genes in different populations. In some populations, a wide spectrum of different mutations in both genes is present, whereas in other groups certain mutations are seen at higher rates. In the Greek population, specific mutations in BRCA1 account for 71% of all mutations detected in both BRCA1 and BRCA2 genes. This study aimed to determine the prevalence of BRCA1 mutations in a Greek cohort of ovarian cancer patients, selected for a strong family history, early age of onset or metachronous breast cancer. 81 patients with ovarian cancer were screened for all the BRCA1 mutations previously identified in the Greek population. Of these, 24 carried a deleterious

BRCA1 mutation (30%), while an unclassified variant was identified in 2 patients (2.4%). Identifying a mutation in the BRCA1 or BRCA2 genes among breast and/or ovarian cancer families is important, as it enables carriers to take preventive measures. Currently, bilateral salpingo-oophorectomy is the most effective way to reduce the risk of ovarian cancer in BRCA1/2 mutation carriers. Further studies are warranted to determine the prevalence of mutations in the BRCA1, as well as in the BRCA2 gene, in both high-risk and unselected ovarian cancer cases in the Greek population.

P06.025 Haplotype analysis of two recurrent genomic rearrangements in BRCA1 gene suggests that they are founder mutations for the Greek population

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The deletion of 4.4kB and 3.2kB identified in exons 24 and 20, respectively, are two of the four most common mutations in the *BRCA1* gene in Greek breast cancer patients. The exon 24 deletion includes both exon 24 as well as the 3' UTR, while the exon 20 deletion starts within exon 20 and extends into intron 20. They have been reported 9 and 6 times, respectively, in unrelated families of Greek origin. In order to characterize these recurrent mutations as founder mutations, it is necessary to identify the disease-associated haplotype and prove that it is shared by all the mutation carriers, suggesting that it occurred only once in a common ancestor.

Genomic DNA was isolated from 19 breast cancer patients and 66 healthy individuals. Ten Short Tandem Repeat (STR's) markers located within and flanking the *BRCA1* gene locus, spanning a 5.9 Mb interval, were used for the haplotype analysis.

The results indicate that all carriers of the exon 24 deletion share a common core haplotype '4-7-6-6-1-3' between markers D17S951 and D17S1299, for a stretch of 2.9Mb. The common haplotype shared by carriers of the exon 20 deletion is '6-7-4-2-6-7-1-3' between markers D17S579 and D17S1299, for a stretch of 3.9Mb. Haplotype conservation ended between markers D17S1299 and D17S800 and between D17S951 and D17S1861.

Both genomic rearrangements in *BRCA1* gene are Greek founder mutations, as all carriers share the same, for each group of mutation carriers, disease-associated haplotype, suggesting the presence of a distinct common ancestor for both of the mutations.

P06.026 Haplotype characterization of BRCA1 gene in North-Eastern Romania

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Introduction: *BRCA1* is major cancer predisposition gene, responsible for a large percentage of hereditary breast and ovarian cancer (HBOC) families. *BRCA1* alleles are ascribed almost completely to 10 canonical haplotypes defined by 14 prevalent SNPs. Two of these haplotypes (H1 and H2) predominate and account for up to 78 % of alleles in Europeans and North-Americans. The frequency and type of *BRCA1* haplotypes varies widely, depending on the geographic and/or the ethnic distributions.

Patients and methods: We investigated 26 patients from 17 unrelated HBOC families in north-eastern Romania. All patients agreed by written informed consent. DNA was extracted from peripheral blood. The entire coding sequence was analysed using dideoxy sequencing. *BRCA1* haplotypes were assigned using 11 common single nucleotide polymorphisms previously described.

Results: We identified five *BRCA1* variations, including novel, recurrent mutations or unclassified variants. We identified seven different haplotypes in our population: five previously described, and two other haplotypes containing an additional SNP, IVS7-34C>T (H1-n and H6-n). Haplotype frequencies appeared to be similar to those reported in other European populations. Five haplotypes were not observed in our population.

Some mutations could be easily assigned to haplotypes. c.2241dupC was assigned to H1, both carriers of the mutation being homozygous for this haplotype. Cys61Gly was observed in association with IVS7-34C>T, and could be assigned to either the H1-n or the H6-n haplotype.

Conclusions: This study lays the groundwork to investigate *BRCA1* haplotypes in eastern populations in more detail, with the aim of comparing groups within Eastern Europe or with western populations.

P06.027 Novel *BRCA2* gene frameshift mutation detected in a woman with multiple primary cancers

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Women with *BRCA2* gene germline mutations have approximately 80% lifetime risk of breast cancer and 10-20% risk of ovarian cancer. In males, *BRCA2* mutations are associated with 14% prostate cancer and 10% male breast cancer risks. In addition the risks of lymphomas, gastric, pancreatic, thyroid and gallbladder malignancies are increased in individuals with *BRCA2* mutations.

In this report we present the germline NM_000059.3:c.8405_8406insC (p.L2803fs) mutation in exon 19 of the *BRCA2* gene, which manifests itself as a premature stop codon. The mutation has not been previously reported at Breast Cancer Information Core (BIC) database.

This mutation was first found in a woman who had been diagnosed with breast cancer at the age of 62 and two years later with stomach and kidney malignant tumours. The patient has a complicated family history: Her twin sister had died at the age of 32 of breast and ovarian cancer and her elder sister had died of breast cancer at 50.

Cascade screening of her close relatives is in process in the time of abstract submission.

P06.028 *BRCA2* N372H polymorphism and breast cancer risk

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Truncating mutations in *BRCA2* gene cause a substantial increase in risk of breast cancer, but such mutations are found only in small number of breast cancer patients. Low penetrance alleles, such as polymorphic variants in strongly predisposing genes, such as *BRCA2*, are candidates for inherited susceptibility to breast cancer. The N372H polymorphism is common variant in *BRCA2* gene that was suggested to affect *BRCA2* structure and function and to moderately increase the risk of breast cancer. The aim of this study was to evaluate the possible association of this polymorphism and breast cancer risk in Macedonian patients. The study included 76 patients with breast cancer and 75 controls from the general population. The N372H polymorphism was screened by single strand conformation polymorphism (SSCP) method. In selected samples, the results were confirmed by direct DNA sequencing. The *BRCA2* N372H allele frequency in patients with breast cancer was estimated at 25.7% and was similar to that observed among controls (28.7%). There was no difference in the *BRCA2* N372H genotype frequencies between patients with breast cancer (52.6% NN, 43.4% NH and 4.0% HH) and controls (48.0% NN, 46.7% NH and 5.3% HH). No difference was detected also when patients were stratified according to the age of diagnosis and family history. In one male patient with breast cancer the N372H polymorphism was found in cis to the *BRCA2* D2723G mutation. In conclusion, our study has failed to support the association between N372H polymorphism and breast cancer risk.

P06.029 Breast cancer genetics: Lobular histology is a negative predictor of *BRCA* mutations

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Lobular breast cancer represents 10-20% of all breast malignancies. Constitutional mutations in cadherin, a cell-adhesion molecule, result in a high risk of lobular breast and diffuse gastric cancers. However, *BRCA-1* and *BRCA-2* are the genes most frequently implicated in hereditary predisposition to breast cancer. Soon after clinical testing for *BRCA* mutations became available, a flurry of publications appeared comparing the histological characteristics of breast cancers in patients with *BRCA-1* vs. *BRCA-2*, vs. patients negative for *BRCA* mutations (*BRCA-X*). The next wave of publications was epidemiological,

whereas the current focus is molecular profiling of breast cancers.

In the interim, histology has been displaced as a consideration in genetic risk assessment. We believe that lobular histology may be as important a *negative* predictor of *BRCA* status as pre-menopausal diagnosis is a *positive* predictor. Although infrequently considered, there is some data from the literature to support this idea. Our experience is as follows:

We have counseled and tested 150 breast cancer patients at significant risk of carrying a *BRCA* mutation, for whom histology was available/adequate and *BRCA* results were unequivocal. Lobular breast cancer was under-represented (8/150, 5.3%). Of these 150 patients:

- 20 (13.3%) had a *BRCA1-2* mutation; three had bilateral disease. All 23 tumors were of ductal histology; none were lobular.
- 130 patients (86.7%) were negative for *BRCA 1-2* mutations. All of the lobular breast cancer patients were in this group.

Histological analysis is an integral part of every cancer patient's evaluation. Why not take it into consideration in genetic risk assessment?

P06.030 Hypermethylation in promoter region of E-cadherin gene is associated with tumor metastasis in breast cancer carcinoma

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Reduced or lost E-cadherin expression is associated with methylation of CpG sites and has a direct relation with breast cancer metastasis status. The authors analyzed the methylation status by bisulfate sequencing and determining its pattern in 10 CpG site (50 cancerous and 50 adjacent normal breast tissues). Data analyses was performed using spearman rank regression statistical methods with SPSS.13 with ($p<0.05$) criteria for assessing significant variations between tumor (50% partial, 44% full methylation) versus normal tissue (76% non-methylated)($p=0.00$). The methylation pattern was also shown to be significantly different in normal and tumor tissues (892, 940 & 879, 887 respectively). An adverse correlation was observed between tumor metastasis and the pattern of methylation in 863, 865, 879, 887, 892, 901, 920 CpG islands while the 873, 918 and 940 islands showed a direct relation (the highest significance was observed in 940, $P=0.074$). No significant linkage was detected between 940 island and chemotherapy. According to the results obtained from present study, it can be concluded that methylation of CpG sites can be seen in normal tissues which means that methylation is not necessarily depends on blocking of gene expression. Significant difference was detected between methylation pattern in normal and tumor tissues in 892 and 940 islands that reveal the specificity of tumor methylation pattern.

P06.031 Evaluation of Methylation in the 5'UTR Promoter Region of *DBC2* Gene in 50 Breast Cancer Individuals and Comparing with the Normal Controls in Iranian Patients

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Breast cancer is one of the most common malignancies affecting women worldwide. Around 10 percent of every woman would be affected through her life. It is caused by a number of genetic and epigenetic factors.

Aberrant hypermethylation of promoter regions in specific genes is a key event in the formation and progression in some cancers. Recently *DBC2* was found to participate in diverse cellular functions, such as protein transport, cytoskeleton regulation, apoptosis and cell cycle control. *DBC2* has been identified as a candidate tumor-suppressor gene for breast cancer. Different studies showed that *DBC2* is being inactivated through epigenetic mechanisms such as methylation in its promoter region, may facilitate proliferation of tumor cells. Inversely

reactivation of it usually suppresses the growth and expansion of the tumors.

There are different techniques to detect the methylation pattern. Methylation specific PCR is an accurate technique. This technique relies on alteration that sodium bisulfate induces in DNA sequences and cause difference between methylated and unmethylated cytosine, by deamination of unmethylated cytosine. Methylation pattern can be detected by using the specific primers set in two separate PCR reactions.

In this study, we evaluated methylation status of *DBC2* in 50 breast cancer samples from 50 Iranian affected women and 5 normal tissues.

The results showed that 23% of patients have methylated bands and 70% are hemimethylated. We also found that 80% of normal tissues are hemimethylated and one normal sample has methylated band.

P06.032 Estrogen receptor α gene mutations in Exon 4 and Intron 1 polymorphisms in a cohort of Breast cancer patients from India

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Purpose: Estrogen receptor (ER) mutations and polymorphisms are possible risk factors implicated in the initiation and development of Breast Cancer (BC). Hence, sporadic BC patients from a cosmopolitan city of South India were assessed for ER α gene mutation in exon 4 and both Pvull and XbaI polymorphisms in intron 1. **Methods:** Genomic DNA was isolated from 310 sporadic BC patients and 210 sex matched healthy controls to evaluate an earlier reported A908G ER α exon 4 mutation by PCR - SSCP and Pvu II and XbaI polymorphisms in intron 1 by PCR - RFLP. **Results:** The expected ER exon 4 mutation was not identified in any of the samples, however, three BC patients showed a novel C975G mutation in the same exon. Results from the analysis of ER polymorphisms indicate that, Pvull PP genotype is significantly associated with BC [OR 1.999, 95%CI -1.099-3.636], while XbaI polymorphism has no association in the cohort studied. **Conclusion:** The functional significance of the novel exon 4 ER α gene C975G nucleotide change identified in 1.2% of the BC patients needs to be assessed. Unlike XbaI genotypes the ER alpha Pvull PP genotype confers a twofold higher risk of BC and may be used as a biomarker for Asian Indians

P06.033 Estrogen metabolism, hormone replacement therapy use and risk of postmenopausal breast cancer

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Introduction: Association between long-term hormone replacement therapy (HRT) use and increased risk of breast cancer is still under debate. Functionally relevant polymorphisms in genes involved in estrogen metabolism may alter exposure to exogenous sex hormones and affect risk of postmenopausal breast cancer. We evaluated associations of common polymorphisms in genes involved in estrogen metabolism, duration of HRT use, and their interactions with breast cancer risk in a case-control study of postmenopausal women.

Patients and methods: We studied 530 incident breast cancer cases and 270 controls matched by age and ethnicity. Genotyping was conducted for COMT Val108/158Met, GSTP1 Ile105Val and MnSOD Val-9Ala polymorphisms by TaqMan® allelic discrimination method. Duration of HRT use was ascertained from a validated questionnaire. Adjusted odds ratios and 95% confidence intervals were calculated using logistic regression analysis.

Results: None of the polymorphisms studied was, by itself, statistically significantly associated with breast cancer risk. HRT use was significantly associated with decreased breast cancer risk. Statistically significant interaction between MnSOD Val-9Ala (pinteraction = 0.049)

and HRT use was observed. The increased risk of breast cancer associated with long-term HRT use was greater among women with at least one variant allele of MnSOD Val-9Ala polymorphism.

Conclusions: Our results suggests that specific polymorphisms in genes involved in estrogen metabolism may modify the effect of long-term HRT use on breast cancer risk.

P06.034 Polymorphism in mitochondrial DNA D loop region of Iranian breast cancer patients: Is it one of the reasons for younger Iranian patients?

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Breast cancer is the most important malignancy for women in the world. In Iran breast cancers incidence is growing and the Iranian patients are 10 years younger than their western counterparts. Defects in mitochondrial function are suggested to contribute to the development and progression of cancer. Mitochondrial DNA (mtDNA) is particularly susceptible to damage by environmental carcinogenesis.

The displacement loop or D-loop is non-coding region in mtDNA and contains essential transcription and replication elements; hence mutation in this region may serve as a potential sensor for cellular DNA damage and cancer development.

Aim of this study is to scan the mutation frequencies in hypervariable regions of mitochondrial D-Loop in breast cancer patients. The results of our investigation are summarized in the following table. When each polymorphism is tested individually using the fisher exact test the frequency of one single-nucleotide polymorphism (SNPs) was found to be significantly different between the breast cancer patients and controls ($p \leq 0.05$).

In this work the fact that the Iranian women who expose to breast cancer are approximately 10 years younger than their western counterparts is investigated. We think that existence of some SNPs in mtDNA in contribution with other genetics and environmental factors may be one of the reasons that cause the mentioned phenomena.

| Fisher's Exact Test p value | Control cases (n=150) | | Breast cancer cases (n=25) | | SNPs |
|-----------------------------|-----------------------|----------|----------------------------|----------|---------|
| | % | Positive | % | Positive | |
| 0.99 | 4 | 6 | 48 | 12 | G16145A |
| 0.99 | 7.3 | 11 | 60 | 15 | C16261T |
| 0.008 | 43 | 64 | 72 | 18 | T16529C |

P06.035 Molecular Characterization of Mesenchymal Stem Cells and The Involvement in Tumor Growth and Metastases.

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Mesenchymal stem cells (MSCs) are present in a variety of tissues like bone marrow stroma. MSCs can differentiate to various lineages includes the osteogenic lineage. Breast metastatic process displays organ specificity, one of the main targets is the bone. The molecular cross-talk between tumor components may enable synergy in its promotion.

We focused on a novel cell adhesion molecule named SVEP1. SVEP1 plays a role in cell adhesion and in breast cancer cells homing to bone niches. We characterize SVEP1 transcriptional regulation in MSCs (MBA15) versus breast cancer cells (DA3).

TSS-SVEP1 promoter is transcriptionally regulated by DNA methylation. By using Methylated Specific PCR method, this region is highly methylated in DA3, but less methylated in MBA15 cells. SVEP1 is transcriptionally upregulated by TNF alpha and Estradiol treatments both in transient transfections, using SVEP1 promoter luciferase reporter plasmid and at the mRNA level. ChIP assay performed with MBA15 cells revealed increased ER alpha and NFkB binding to SVEP1 promoter following TNF alpha or Estrogen treatments. In contrast, increased binding of ER alpha and NFkB was found in DA3 cells stimulated with TNF alpha, but not following Estrogen stimulation. TNF alpha and Estrogen also regulate SVEP1 promoter through affecting its methylation status. We present differential expression of SVEP1 alternative spliced forms in MSCs versus Breast carcinoma cells which in turn can add another level of regulation.

SVEP1 and its spliced forms can be used as molecular diagnostic marker to isolate tumor initiating as well as metastatic cells and be potential therapeutic target.

P06.036 The correlation between E-Cadherin protein and estrogen receptor alpha C promoter methylation, in pathogenesis of Iranian patients with breast cancer

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Breast cancer is the leading cause of cancer among women. Most of breast tumors originate from mammary epithelial cells and are estrogen dependent. Estrogen exerts its effects through at least two nuclear receptors, ER α and ER β , which have an important role in proliferation and differentiation of normal epithelial cells in breast tissue.

It has been shown that, receptor positive cells in breast cancer can become estrogen negative. However the exact mechanism of estrogen receptor negativeness in breast cancer needs to be clarified. Promoter methylation at the 'CpG islands' in ER α has been investigated as an appealing mechanism of gene silencing.

On the other hand, E-cadherin is a cell-cell adhesion protein, with a prominent role in epithelial differentiation. Data suggest that E-cadherin is also a tumor suppressor in breast cancer.

In this research, we investigated the role of ER α C promoter methylation in Iranian patients with breast cancer, by Methylation Specific PCR in 40 blood and tissue samples in comparison with the percentage of E-Cadherin obtained by histopathological data from patients' medical records. Primers were selected to be specific for a known CpG island in C promoter of ER α , either for methylated or unmethylated status.

Finally Statistical analysis were done between E-Cadherin percentage and the methylation patterns of ER α C promoter and a significant correlation was found (p-value =0.04). This result was in concordance to those reported in the literature, which show a role of ER α methylation and E-Cadherin protein percentage in pathogenesis of Iranian breast cancer patients.

P06.037 Homozygous BUBR1 mutation and susceptibility to multi-site gastrointestinal neoplasia

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A 67 year old male was diagnosed with an ampulla of Vater adenocarcinoma at age 34, followed by multiple adenomas and carcinomas of the stomach and colon in a setting of an unrecognized form of mosaic variegated aneuploidy (MVA). He was found to have a very high degree of premature chromatid separation (PCS) and chromosomal abnormalities. A homozygous mutation causing an intronic de novo splice site was found in BUBR1, a cause of MVA. All heterozygous relatives had intermediate levels of PCS. Functional analyses indicated that homozygosity for this mutation causes a dramatic reduction in the amount of functional BUBR1 protein. This down-regulation caused mitotic abnormalities: PCS, aneuploidy, cell cycle dysfunction, and multiple cellular abnormalities (micronucleated cells, centrosome amplification). Immunohistochemistry revealed a decreased amount of BUBR1 in both normal and tumoral tissues in colon and stomach from proband compared to controls. Loss of functional APC (Adenomatous polyposis coli) is one of the major causes of colorectal cancer. We demonstrated that the interaction between the APC protein and BUBR1, previously reported as crucial for APC activation, was weaker in cells from the proband. Stable expression of BUBR1 cDNA in cells of the proband rescues the PCS phenotype, the cell cycle abnormalities, and the interaction between APC and BUBR1. Screening of a control population revealed that the mutation is a rare variant. This makes BUBR1 a strong candidate for mutation screening in cases of multiple gastrointestinal cancers and highlights the possibility of deregulation of SAC genes as a cause of gastrointestinal cancers.

P06.038 The inverse ratio of BRCA1 and BRCA2 gene expression in ovarian cancer tissue and in tumour environment.

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We investigated BRCA1 and BRCA2 gene expression in samples of ovarian cancer tissues, in tissues of tumour environment and distant from tumour regions. The BRCA1/2 gene expression was determined comparative to expression of GAPDH gene by RT-PCR using Applied Biosystems TaqMan kits. The BRCA1 gene expression in tumour comparative to distant region was decreased in more than half of samples. Approximately the same result was obtained for BRCA2 although simultaneous decreasing of both gene expression was characteristic only for 30% of patients. In tumour environment tissue BRCA1 gene expression was decreased in comparison with tumour tissue for 60% of cases. For the same cases BRCA2 expression ratio in tumour and environment tissues was inverse comparative to BRCA1 expression ratio. In the cases when BRCA1 expression level was less in tumour than in environment tissue BRCA2 expression was inverse also. Thus for the first time it is revealed BRCA1 and BRCA2 participation in tumour and environment tissue interplay. This is important in the light of data about microenvironment influence on the processes of malignant tumor onset and development and may be necessary for therapy creation.

P06.039 Is mitochondrial genome cell's Achilles' heel of carcinogenesis?

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Objectives: Investigation of the role of mtDNA inherited polymorphisms in breast cancer and endometrial adenocarcinoma development.

Methods: mtDNA D-loop sequence and haplogroup distribution analysis were performed in the cohorts of cancer patients, centenarians and woman with cancer free history. Two tailed non-directional Fisher-Irwin test has been used to verify the mutation probability difference between cohorts and general Polish population.

Results: In endometrial cancer cohort haplogroup H is under-represented if compared with healthy population ($p = 0.001$). Negative Predictive Value of T7025C RFLP test=0.581 and Relative Risk Reduction=0.725. Inheritance of 7028T results in increased risk of endometrial cancer development as high as 350% in comparison to 7025C. Other mtDNA polymorphisms in particular 16223C ($p = 0.005$), 16126C ($p = 0.025$) and 207A ($p = 0.027$) are abundant in endometrial cancer population.

Breast cancer patients in comparison to cancer free control group abundantly carry 10398G ($p < 0.001$). This polymorphism is cancer development factor with odds ratio=9.510 and RR=7.576. A10398G RFLP test has high sensitivity=0.769 and specificity=0.740. Moreover in breast cancer population A1811G and A4529T are overrepresented ($p=0.001$) if compared with centenarians.

Conclusions: 10398G polymorphism inheritance may predispose to breast cancer development, while 1811A and 4529A may protect from it. Carriers of T7028C seem to be protected for endometrial adenocarcinoma development, but inheritance of 16223C 16126C and 207A is a risk factor. mtDNA polymorphisms establish a specific genetic background for cancer development and may enable selection of populations at high cancer risk and support the process of prevention and early diagnosis.

P06.040 Nature of untargeted mutations in the polymerase-tautomer model of ultraviolet mutagenesis

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Delayed and untargeted mutations are two features of genomic instability, the main cause of cancer. It is common hypothesis that they induce mutations at non damaged sites. The polymerase-tautomer model for ultraviolet mutagenesis is based on formation of rare tautomeric bases in *cis*-syn cyclobutane pyrimidine dimers and DNA bases [1], and that fact that during error-prone or SOS synthesis the induced DNA polymerase inserts canonical bases opposite the dimers in a complementary way in contrast to uncomplementary one in the conventional models [2]. Potential mutagens correspond to 5 basic types of rare tautomeric conformations of thymine and adenine [1] and 7 of cytosine and guanine. Potential untargeted mutation is rare tautomer of guanine - cytosine pair, when protons in first and second hydrogen bonds were simultaneously sent to the partners on hydrogen bonds. They will be stable and can cause transition or homologous transversion. In the case of closely located dimers in the both strands, the adenines or guanines that are in rare tautomeric states can be a source of untargeted mutations. The third source is bases in rare tautomeric state localized in small vicinity of dimers. They may result in transition, transversion and frameshift mutations under error-prone or SOS synthesis.

1. H. A. Grebneva. *J. Mol. Struct.* - 2003. Vol. 645. P. 133-143.
2. H. A. Grebneva. *Environ. Mol. Mutagen.* - 2006. Vol. 47. P. 733-745.

P06.041 Germline mutations of the CBL gene define a new genetic syndrome with predisposition to juvenile myelomonocytic leukemia (JMML)

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Background: *CBL* missense mutations have recently been associated with juvenile myelomonocytic leukemia (JMML), an aggressive myeloproliferative and myelodysplastic neoplasm of early childhood characterized by excessive macrophage/monocyte proliferation. *CBL*, an E3 ubiquitin ligase and a multi adaptor protein, controls proliferative signaling networks by downregulating the growth factor receptor signaling cascades in various cell types.

Methods and results: *CBL* mutations were screened in 65 patients with JMML. A homozygous mutation of *CBL* was found in leukemic cells of 4/65 (6%) patients. In all cases, copy neutral loss of heterozygosity of the 11q23-qter chromosomal region, encompassing the *CBL* locus, was demonstrated. Three of these 4 patients displayed additional features suggestive of an underlying developmental condition. A heterozygous germline *CBL* p.Y371H substitution was found in each of them and was inherited from the father in one patient. The germline mutation represents the first hit, with somatic loss of heterozygosity being the second hit positively selected in JMML cells. The 3 patients display a variable combination of dysmorphic features, hyperpigmented skin lesions and microcephaly that allow us to tentatively delineate a "CBL syndrome". Learning difficulties and postnatal growth retardation may be part of the phenotype.

Conclusion: We report germline mutations of *CBL* in 3 patients with JMML, confirming the existence of an unreported inheritable condition associated with a predisposition to JMML.

P06.042 Mutation screening of *CDKN2A* gene in malignant melanoma families from the Slovakia

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Germline mutations in the *CDKN2A* gene have been linked to inherited predisposition to malignant melanoma. Variable frequencies of *CDKN2A* germline mutations were reported in different populations. High percentage of the mutations were observed only once and are family specific. There is also an increased risk of pancreatic cancer in a subset of families with mutations.

We have screened 32 high-risk melanoma families from Slovakia using a direct sequencing technique. There was examined the entire coding region of the p16 and p14 gene, including exons 1 α , 1 β , 2 and 3, flanking exon/intron junctions, and portion of the 5' untranslated (UTR) region of the gene.

Analysis of *CDKN2A* gene showed presence of 2 novel missense mutations, one mutation c.100G>C (Ala34Pro) was identified at exon 1 α in a two different melanoma families. The second mutation c. 150G>C (Gln50His) was detected in one family at the same exon. Both substitutions was not previously described, and have undetermined pathogenic effect. There was also observed common polymorphism Ala148Thr and frequent variants C/G in the 3'UTR at the position 29 after stop codon.

In summary, within 32 Slovak melanoma families, we identified 3 families (9,4%) with two types of novel *CDKN2A* mutations, which has not been reported yet. These new substitutions will need additional examination of functional impact to gene *CDKN2A*.

P06.043 Constitutional genomic imbalances in children with cancer and congenital anomalies

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Congenital malformations are present at higher frequencies in children with cancer than in age-matched controls. Constitutional genomic imbalances may underlie these clinical manifestations. We set out to identify such anomalies using a high resolution genomic profiling approach. To this end, twenty-seven patients with a pediatric malignancy and a congenital anomaly were analyzed on a SNP array platform (Affymetrix). In two of these patients submicroscopic imbalances were found. The first patient was a girl with acute lymphoblastic leukemia (ALL) and polysyndactyly of the toes. She harbored a intragenic germline deletion of the *THADA* gene. This gene is considered to play a role in apoptosis. Interestingly, *THADA* deletions have previously been encountered in sporadic paediatric ALLs. In the second patient, a girl with embryonal rhabdomyosarcoma and a congenital heart defect, we detected a microduplication of the chromosome 22q11.2 region. This micro-duplication appeared to be inherited from the mother who suffered from meningioma. This same region is typically deleted in patients with velocardiofacial syndrome. In addition, a 22q11.2 microduplication syndrome has recently been recognized. As yet, however, these syndromes have not been associated with tumor predisposition. Our results indicate that high-resolution genomic profiling of patients with a childhood malignancy and a congenital anomaly may hold promise as an approach to identify novel cancer predisposing genes.

P06.044 Deregulation of proapoptotic genes in skull base chordoma

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Chordoma is a rare tumour arising from remnants of notochord, characterized by local invasiveness and variable tendency to recurrence. Given the implication of apoptosis in notochord regression, we studied in 21 tumours the expression by RT-PCR of 8 proapoptotic genes mapping in 1p36, region showing loss of heterozygosity in most chordomas (83%). *TNFRSF8* and *TNFRSF9* were found to be differently expressed in 45% of tumours in comparison to the control nucleus pulposus, while *DFFA*, *DFFB*, *CASP9*, *TNFRSF1B*, *TNFRSF14* and

TP73 were occasionally observed differently expressed in comparison to the control. The expression profile of each tumour was compared to that of the control and none of them but one, overlaps the control expression profile. As the apoptotic pathway mediated by FAS-FASL is involved in notochord regression, we studied their expression in 34 tumours and in 3 chordoma cell lines. Since most chordomas expressed FAS but not FASL, we treated a chordoma cell line, expressing FAS but not the ligand, with the soluble FASL at different times and concentrations. By FACS analysis following apoptotic assay, we observed apoptosis induction after soluble FASL administration. The expression of TP53, involved in the intrinsic apoptotic pathway mediated by FAS-FASL, was also investigated by Real-Time PCR in 23 chordomas: upregulation was detected in 53% of tumours. A correlation study between the expression profiles of proapoptotic genes and the patients' follow up will be carried out to search for prognostic markers, while the possible pharmacological effect of FASL will be investigated in primary chordoma cell cultures.

P06.045 Detection of BCR-ABL breakpoints in Iranian patients with Chronic Myelogenous Leukemia

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Chronic Myelogenous Leukemia (CML) is a myeloproliferative disorder characterized by increased proliferation of the granulocytic cell line without the loss of their capacity to differentiate. Translocation between the BCR locus on chromosome 22 and the ABL locus on chromosome 9 generates a chimeric gene, BCR-ABL. The protein product of this chimeric gene exhibits an altered tyrosine kinase activity which is implicated in the progression of CML.

In this study, peripheral blood samples from the patients admitted to Kariminejad- Najmabadi genetic center were subjected to RNA isolation and cDNA synthesis. To achieve maximal sensitivity nested PCR protocol was used and a housekeeping gene was considered as an internal positive control.

We have studied 715 patients and the following results were obtained: as from 715 CML patients, 266 of them were positive for t(9;22) (q34;q11), and among them 151 (56.76%) had 'b2a2', 113 (42.48%) 'b3a2' and 2 (0.75%) had the 'e1a2' breakpoints.

Most of our patients showed 'b2a2' fusion gene (56.7%), while the remaining showed one of the transcripts of 'b3a2' or 'e1a2'. The rate of coexpression of the 'b3a2' and 'b2a2' was 5%. In contrast to other reports, we did not see any coexpression of p210/p190. According to this experiment 'b2a2' breakpoint is more common among Iranian population which is in contrary with other literature, indicating 'b3a2' breakpoint as the most common one. To be able to find the most common breakpoint in Iranian population we need to continue the experiment in a larger scale and among different ethnics.

P06.046 Research and Clinical Importance of Duplications in Various Chromosomal Regions in Addition to Philadelphia Chromosome in Chronic Myeloid Leukemia

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It was aimed to investigate the chromosomal aberrations in Chronic Myeloid Leukemia (CML) and particularly the aberrations in the chromosomal regions, which carried 67 genes pertaining to oncogene, transcription factor, signal transmission, cytokine, immune system, tumor suppressor and apoptosis, in addition to Philadelphia (Ph+) chromosome by MLPA method and to compare them with clinical parameters. In this study, we were investigated with MLPA method in 48 patients, who were diagnosed with chronic phase CML or were under treatment process and in 15 healthy controls. The obtained results were compared both among each other and with clinical parameters and their effects on survival were evaluated. Seventy patients were male whereas 31 of them were female. The median age was 43 (20-74). Duplication was detected in FGFR1 gene of 2 patients, IMPDH1 gene of 4 patients, PMS2 gene of 1 patient, NFKB1 of 5 patients and

LMO2 gene of 1 patient. In multivariate analysis, it was observed that only the duplications in IMPDH1 and FGFR1 genes were the most important factors that affected event-free survival (p=0.028). Duplications in 4 genes in CML patients, who used imatinib, were detected for the first time. The duplications in IMPDH1 and FGFR1 genes, located in chromosomes 7 and 8 and being in charge of signal transmission, particularly had negative effects on event-free survival. In conclusion, this study puts forward that chromosomes 7 and 8 should particularly be investigated in more detail in addition to Ph+ chromosome in the determination of prognosis and selection of treatment alternatives.

P06.047 The isotherm of joint binding of different types of ligands with DNA

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Cis-DDP is frequently used as an anti - tumor drug. That is why it is very important to investigate the binding parameters of the cis-DDP with DNA. The special method has been developed for study of cis-DDP bound to DNA irreversibly. The essence of the proposed method is to obtain the information about the irreversible binding of cis-DDP with DNA on the basis of isotherm of adsorption of the reversibly binding ligand (EtBr) on DNA in presence of cis-DDP. The adsorption of ligands has been considered in case of small amount of both irreversibly and reversibly binding matter. With taking into account the existence of two regions with different binding features in DNA, the adsorption isotherm and dispersion has been calculated. The isotherm of adsorption of EtBr on DNA has a linear form in Scatchard coordinates at low degrees of occupation. It was shown that irreversible binding of cis-DDP on DNA results to transformation of the linear in Scatchard coordinates isotherm of adsorption into non-linear isotherm. It was shown also that comparison with experimental isotherm of reversibly binding ligand permits to estimate such important parameters as binding constant, number of binding sites on DNA per ligand, and the fraction of DNA molecules have been changed their features under influence of irreversible binding of cis-DDP with DNA.

P06.048 Prognostic significance of interstitial del(14)(q) with deletion IGH(C) in patients with CLL.

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Introduction: Deletions of long arm of chromosome 14 are recurrently observed in malignant B- cells and are often detected in patients with CLL. We wanted to answer following questions:

What is CLL biology and genetics with deletion of IgH (C)?

Which characteristics have patients with CLL and patients with this change?

Does del IgH (C) have an influence for patient's surviving?

Methods: We have identified patients with deletion of IgH (C) by the method FISH with LSI IGH Dual color, Brak apart probe (Vysis) and consequently, we have examined these patients by the method arrayCGH for specification of size of this deleted region. Array CGH results were validated by FISH with BAC probes 14q24.1(ZFP36L1), RP11-204K16sg (179,9 Kb) a 14q32.1, RP11-79J20so (168,2 Kb) (Pentagen Ltd.).

Results and Conclusion: We have identified interstitial del(14)(q24.1q32.33) in 5 patients by the method arrayCGH. We have found out the extent of IgH (C) deletions as well as the additional chromosomal aberrations.

Deletions of IgH (C) refer to assumption of interstitial deletions of 14q with breakpoint located proximally from E_μ (enhancer). Del(14)(q24.1q32.33) are occurring recurrently and in this region gene ZFP36L1 is located, which possibly plays a role in disease pathogenesis.

Correlation of incidence del(14)(q24.1q32.33) with surviving in 5 patients refers to the fact, that isolated del(14)(q24.1q32.33) was associated in patients with good surviving independently of age, clinical phase, IgH (V) mutation status.

That all points to: participation on disease development, but not as a significant prognostic marker associated to a bad prognosis.

P06.049 *AHI1* Gene Expression Levels and *BCR-ABL1* T315I Mutations in Chronic Myeloid Leukemia Patients

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With the availability of molecular monitoring of BCR-ABL1 and the use of imatinib, therapy in chronic myeloid leukemia (CML) now targets its molecular pathology. While in time a group of patients would acquire resistance to imatinib, alternative tyrosine kinase inhibitors have been developed in this aspect. In this study, 83 samples taken at different time points from 38 CML patients; were subjected to gene expression analysis of *AHI1*; a novel gene that is thought to have a role in both BCR-ABL1 mediated leukemic transformation via the JAK/STAT pathway and response to tyrosine kinase inhibitors. In the same samples, the presence of T315I mutation was investigated. Only one patient (2.63%) harbored the T315I mutation. While no significant difference in *AHI1* expression was observed between newly diagnosed CML samples and non-CML controls; CML samples under imatinib therapy had levels significantly higher than both newly diagnosed samples and controls. In the first 6 months of imatinib therapy, *AHI1* expression was found to increase, then decrease gradually in time. There was no significant difference between imatinib responders and non-responders, while cases on dasatinib had significantly lower *AHI1* levels. It is proposed that the change in *AHI1* expression during CML therapy might be under the control of mechanisms independent from BCR-ABL1. *AHI1* mediated signaling could be better understood by analyzing *AHI1* gene expression levels in a greater number of patients and concurrently investigating the JAK/STAT and Src family kinase pathways.

P06.050 First evidence for digenic inheritance in hereditary colorectal cancer by mutations in the base excision repair genes

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Biallelic mutations in the base excision repair gene MUTYH are responsible for variable recessively inherited phenotypes of polyposis. Beside MUTYH, the proteins OGG1 and MTH1 (or NUDT1) are also involved the repair of 7,8-dihydro-8-oxoguanine (8-oxo-G). So far, previous studies only found missense mutations in either MTH1 or OGG1 with additional heterozygous mutations in MUTYH.

To investigate the role of a defective 8-oxo-G repair we performed a germline mutation screening in the genes MUTYH, OGG1 and MTH1 in 81 patients with a clinical phenotype ranging from attenuated or atypical adenomatous polyposis coli including hyperplastic polyps to HNPCC syndrome without microsatellite instability in their tumours and no germline mutation detectable in mismatch repair genes MLH1, MSH2 and MSH6 or APC.

We describe here the first pathogenic germline mutation in OGG1, a splice site mutation affecting exon 1 which was inherited from the father, in combination with a maternal MUTYH missense mutation p.Ile209Val in a female patient with synchronous colon cancer at age of 36 years pointing towards digenic inheritance for colorectal cancer predisposition.

Monoallelic missense mutations in MTH1 (3), OGG1 (2), or MUTYH (3) were identified in 11 patients, of those, four mutations were novel. Our findings indicate that other genes of the 8-oxo-G repair beside MUTYH might be involved in hyperplastic polyps, attenuated polyposis and colorectal cancer predisposition, either as single heterozygote or double heterozygote mutations.

P06.051 Genetic biomarkers spectrum and molecular tools utility in metastatic colorectal cancer diagnostics in patients of Polish origin.

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K-RAS gene encodes G-protein involved in EGFR-induced cell signaling. Mutations in K-RAS are found in about 40% of metastatic colorectal cancers and are connected with lack of response for novel therapeutic agents targeting the EGFR signal pathway. Estimation of mutational status of K-RAS is necessary for the selection of patients, who should be treated with targeted therapies, however only subset of

patients without K-RAS mutation are good responders for the agents. This is why mutations in others genes involved in EGFR signal pathway (e.g. BRAF and PIK3CA) are necessary to investigate in routine molecular diagnostics. Another issue is lack of validated and standardized methods for mutation detection in the highly heterogenic material isolated from paraffin blocs, which may lead to false negative or false positive results.

The aim of our study was: 1) to analyze the mutational status in K-RAS, BRAF and PIK3CA in group of 300 Polish patients with metastatic colorectal cancer 2) to compare preanalytic and analytic phases tools in order to propose recommendations for molecular testing. Molecular analysis is still proceed, thus final conclusions and summaries will be presented after completion of the study.

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P06.052 Involvement of distinct molecular mechanisms in development of proximal and distal sporadic colorectal cancers

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Published data suggest existence of two histologically and genetically distinct subtypes of colorectal cancers in regards to the site of origin, with implications for the tumor prognosis and employment of therapeutic regimens. Our study aims at comparing the clinicopathological and molecular profiles between colon cancers located proximally and distantly to the splenic flexure among patients from the Republic of Macedonia. A total of 420 sporadic CRC were evaluated for the presence and extent of microsatellite instability and loss of heterozygosity using fluorescent multiplex PCR and capillary gel electrophoresis. Patients with a history of CRC in the family were excluded from the study. The obtained results indicate that significant majority (70%) of CRC detected in the Republic of Macedonia are located distally to the splenic flexure, develop at an older age (61.05ys) predominantly in female population ($p=0.0159$, OR=1.742 CI 95% = 1.11-2.74) and show loss of heterozygosity at 18q ($p=0.0454$, OR=1.72, CI 95% = 1.0-2.93), as well as tendency towards allelic loss at 8p, 1p and 5q. Proximal tumors develop in younger (59.45 ys) men and show significantly higher frequency of microsatellite instability ($p=0.000037$, OR=7.67, CI 95% = 2.69-21.87) in comparison to the distal CRCs. Our data clearly support the hypothesis for involvement of different molecular mechanisms in development of proximal and distal sporadic colorectal cancers, ongoing prospective studies should elucidate the possible prognostic differences as well as the predictive value of the underlying molecular mechanisms for establishment of effective therapeutic approach.

P06.053 The Use of Multifactor Dimensionality Reduction to Detect Epistasis Among Potential Causal Genes of Colorectal Cancer

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Aims/hypothesis. Colorectal cancer (CRC) is a complex genetic disease, which results from interactions between multiple genes and environmental factors without any single factor having strong independent effects. This study was done to identify gene to gene interactions which could be associated with the risk of CRC. The multifactor-dimensionality reduction (MDR) method has been shown to be effective for detecting and characterizing gene to gene interactions in case-control studies with relatively small samples.

Methods. We genotyped 14 different polymorphisms in 9 candidate genes for disease in 180 unrelated CRC patients and 60 control subjects. We analyzed gene to gene interactions among 14 polymorphic loci using the MDR method.

Results. We identify a tree-locus model between DCC (rs714), ACE (rs4291) and IGF2 (rs680) that have a maximum CV consistency of 7 out of 10, and a four-locus model between ACE(rs4646994), VDR (rs2228570), MTHFR (rs1801133) and IGF2 (rs680) that have a maximum CV consistency of 5 out of 10.

Conclusions/interpretation. Using the MDR method, we showed a three-locus interaction between the DCC, ACE and IGF2 among 9 candidate genes of CRC. The determination of such genotype combinations contributing to CRC could provide a new tool for identifying high-risk individuals.

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P06.054 Association of polymorphisms in the hMLH1 gene with sporadic colorectal cancer risk

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Mismatch repair (MMR) genes are among of the most important genes associated with colorectal cancer. Numerous polymorphisms (mainly SNPs) have been identified for DNA repair genes, although no study of association between single nucleotide polymorphism (SNPs) of hMLH1 gene and Iranian sporadic colorectal cancer (SCRC) is available. To address this issue, we examined 4 reported single-nucleotide variants that have rarely been verified in a population-based study to identify SNPs and the genotype-phenotype association in Iranian populations of 100 healthy individuals and 174 SCRC patients. We extracted the genomic DNA from the blood of these individuals and used Pyrosequencing technology to determine these SNPs. All 4 single-nucleotide variants of hMLH1 gene examined in this study were identified as SNPs. Whereas rs1799977 and rs4986984 did not seem to affect SCRC risk, rs2308317 (OR, 6.085; 95% CI, 2.706 - 13.681; $P < 0.001$) and rs2020873 (OR, 6.026; 95% CI, 2.261 - 16.062; $P < 0.001$) did show statistically significant differences between cases and controls

P06.055 Multiple primary malignancies and subtle mucocutaneous lesions in patient with Cowden syndrome (CS)

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Cowden syndrome (CS) is a rare autosomal dominant disorder characterized by multiple benign polyps called hamartomas and an increased risk of developing tumours, mainly breast, thyroid and endometrial carcinoma. Almost all (99%) CS patients present mucocutaneous lesions by their thirties. Macrocephaly, Lhermitte-Duclos disease (LDD), gastrointestinal polyps and goitre are another features commonly presented in CS patients.

We examined a 58 year-old woman with subtle mucocutaneous lesions and multiple primary malignancies. She had been followed up for a period of 23 years, when she developed gradually thyroid, ovarian, stomach, colon and benign meningiomas. Mutation analysis of the *PTEN* gene revealed a novel germline mutation (c.438delT, p.Leu146X) in the patient (submitted).

Although breast and thyroid cancer are predominant malignancies in CS, it should be emphasized that benign and/or malignant tumours may also develop in GI tract from pre-existing polyp, in genitourinary tract or in brain. Most papers dealing with cancer in CS patients do not show the lifetime risk of malignancy in CS because of the relatively young age of the patients. We suppose that meningioma and GIT cancers, although occurring rarely, should be a part of the definition of CS and the physician who might encounter the disease should be aware of this neurological and/or GI manifestation.

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P06.056 Beta-catenin gene mutation analysis in Saudi diagnosed Endometrial Cancer Patients

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Endometrial cancer (EC) is the most frequently diagnosed gynecological malignancy reported worldwide with an estimated incidence of 15-20 cases per 100,000 women per year. Beta-catenin (CTNNB1) gene

plays a role in regulating normal cell growth and behavior. Mutation of CTNNB1 gene has been reported in colon carcinomas, medulloblastoma and ovarian carcinoma. The aim of this study was performed to investigate the CTNNB1 gene mutation in EC pathologically diagnosed cases. Presence of these molecular markers may elucidate better understanding of CTNNB1 gene behavior in our EC diagnosed patients. Genomic DNA was extracted from paraffin embedded sections of tumor and normal tissue from 73 patients with EC (59 endometrioid and 14 non-endometrioid). CTNNB1 mutations in exon 3 were assessed with direct DNA sequencing using ABI 3130xl Genomic Analyzer. CTNNB1 mutations were identified in 20 ECs (33.9%), all of them endometrioid carcinomas (20 of 59; 33.9%). The results of this study may add more insight into the molecular mechanisms of CTNNB1 gene in endometrial tumorigenesis process occurs in Saudi Arabia patients.

P06.057 The Determination of FAS and TNF- α and IL-6 Gene Polymorphisms in Early Stage Mycosis Fungoides

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Mycosis fungoides (MF) is the most common of primary cutaneous lymphomas. Although, environmental and occupational exposure to solvents and chemicals has been implicated in the etiology of the disease, the cause and risk factors of MF are still unclear.

In this study, we have aimed to determine the FAS (-670 A > G, rs1800682) and TNF- α (-308 G > A, rs 1800629), interleukin-6 (IL-6) (-174 G > C, rs1800795) gene polymorphisms in early stages of MF patients (n=25) and compared them with control subjects (n=95) to clarify the potential role of these polymorphisms in MF. We found significant increases in IL-6 CC genotype (Odds ratio: 36.55, $p < 0.01$) and C allele frequency (Odds ratio: 3.35, $p < 0.01$) in MF group. Nevertheless, not any significant change determined for FAS and TNF- α polymorphisms.

P06.058 Analysis of DICER1 gene dosage in hematological malignancies

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Ribonuclease Dicer is the key enzyme required for the biogenesis of microRNA and is essential for both mammalian development and cell differentiation. Recent evidence indicates that Dicer may also play an important role in cancer. Although the expression patterns of DICER1 in different types of tumors were studied so far, the influence of DICER1 gene dosage on the progress of cancer remains largely unexplored. Therefore, in this study we investigate whether DICER1 gene dosage is altered in patients with hematological malignancies.

Genomic DNA was extracted from peripheral venous blood and bone marrow. For gene dosage analysis, real-time PCR quantification of DICER1 and HFE1 as a reference gene was carried out, using SYBR Green I as a dsDNA saturating dye. Relative DICER1 gene dosage was calculated using the two standard curve method.

We included 114 patients with hematological malignancies (clinically confirmed MPNs, AMLs or CMLs) and 78 apparently healthy individuals as a control group. We found no difference in DICER1 gene dosage relative to the reference gene in patients with hematological malignancies. Additionally, HFE1 gene dosage was not altered in any tested individual.

We conclude that DICER1 gene dosage is not altered in the majority of patients with MPNs and AMLs/CMLs. Any potential change in the expression of DICER1 in these types of malignancies is therefore not caused by the alteration of gene dosage. The copy number polymorphisms of both DICER1 and HFE1 genes may be too rare in the general population to be detected in the limited number of individuals.

P06.059 Analysis of frequency NFKBIA gene polymorphism (rs696) in Polish patients with differentiated thyroid cancer

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Thyroid carcinomas are the most often carcinomas of endocrine system with growing up frequency. The most often occurs papillary and follicular thyroid cancer, which belong to group of well prognoses tumors with slow progress and low benignity. Very serious problem are recurrences and regional or remote metastasis. Progression from well differentiated thyroid cancer to malignant anaplastic carcinoma is possible also.

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 thyroid gland tumors development and allow to better diagnosing.

We analyzed c.*126G>A polymorphism (rs696) in NFKBIA gene. Groups of 273 patients with differentiated thyroid cancer and 186 individuals from population group were examined. Sequence variants were determined by pyrosequencing.

There were observed differences in allele and genotype frequencies. In patients with thyroid cancer allele G was present with frequency 0,579 and allele A with frequency 0,421, compared with 0,521 and 0,478 in population group respectively. The differences were more significant when considerate men and women separately. Allele G in males with DTC was observed with frequency 0,651 comparing with males population control 0,533; allele A with frequency 0,349 in patient males and 0,467 in males population.

Regarding lower frequency of the disease in males, detected differences may indicate on association of allele G with thyroid cancer risk.

P06.060 The association between CDKN2A/p16 aberrant DNA methylation with few risk factors such as obesity and occupational airborne exposures in esophageal cancer patients

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There are some risk factors for increasing of squamous cell carcinoma of esophagus such as obesity and occupational dust exposure which we tested whether these factors could also affect aberrant DNA methylation. For this reason, we studied for methylation at the CDKN2A/p16 gene promoter 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 were used to assess association of promoter methylation with bio-pathological, clinical and personal information data, including obesity and airborne exposures. The results showed that in 27.2% (12 out of 44) of tumor samples, Methylation at the CDKN2A/p16 gene promoter was detected but none of the non-tumor tissues exhibited the aberrant methylation. Moreover, the results confirmed previously described significant association with low tumor stage ($P=0.002$); in addition, we found that obesity ($P=0.001$) and occupational exposure ($P=0.008$) were both significantly associated with CDKN2A/p16 promoter methylation. This study provides evidence that obesity and occupational exposure increase the risk of developing esophageal cancer through an enhancement of CDKN2A/p16 promoter methylation.

P06.061 Detection of EGFR mutations from cytologic specimens of Non-small cell lung cancer in Slovak Republic

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Objective: Lung cancer is the leading cause of death in the Slovak Republic. Lung cancer includes small cell lung cancer and non-small cell lung cancer. NSCLC accounts for approximately 80% of lung cancer. Chemotherapy for NSCLC remains marginally effective with a 5-year overall survival in 5-18%. EGFR tyrosine kinase inhibitor gefitinib was approved in Slovak Republic for the treatment of NSCLC. Gefitinib is a selective EGFR inhibitor that binds to the ATP binding pocket of the kinase domain and blocks downstream signaling pathways. Mutations of the EGFR, in-frame 15bp deletional mutation (delE746_A750) in exon 19 and L858R in exon 21 correlated with a clinical responsiveness to EGFR tyrosine kinase inhibitors.

Material and methods: We established methods for detecting delE746_A750 and L858R mutations from cytologic materials by a high resolution melting analysis and a mutant-enriched PCR. The results were compared with direct sequencing. We analyzed 60 archived cytologic specimens from patients with NSCLC.

Result: Mutations of EGFR were detected in 13 cases. In-frame deletion in exon 19 were detected in 15% and L858R mutation in exon 21 in 9,4% cases. The results of the mutant-enriched PCR and high resolution melting analysis were consistent. However one mutant case in exon 21 and two cases in exon 19 were not detected by direct sequencing. The pathological findings of these specimens showed a 5% fraction of tumours cells.

Conclusion: Our results indicated that mutant-enriched PCR and high resolution melting analysis are the same sensitive methods for the detection of EGFR mutations from cytologic specimens.

P06.062 Genetic analysis on EGFR prior treatment with tyrosine kinase inhibitors on patients with non-small lung cancer (NSCLC)

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Aim: To develop methods for EGFR mutation screening with high sensitivity for selection of lung cancer patients who will benefit from treatment with tyrosine kinase inhibitors (TKIs).

Background: Lung cancer is the most common cancer related cause of death in the western world, including both men and women. The mortality is high, less than 15% are living 5 years after diagnosis. The main reason for the poor prognosis is that the majority of patients are diagnosed with metastasis. Therefore, it is of great importance to have effective treatment to decrease the mortality among these patients. During the last couple of years new ways of treatment, using tyrosine kinase inhibitors (Gefitinib, Erlotinib), have successfully evolved. However, best drug response is observed in patients with specific mutations within the gene encoding the Epidermal Growth Factor Receptor (EGFR). This gene plays a major role in the regulation of tumour cell proliferation. Therefore, genetic analysis of the tumour DNA should be performed prior treatment of patients.

Method: We will develop methods for specific mutation analysis of exon 19 and 21 in the EGFR gene. This includes fragment analysis on ABI 3130 and quantitative Taqman based PCR. Genetic analysis of genes downstream in the EGFR signalling pathway, including KRAS, BRAF and PIK3 will also be performed with these techniques to search for additional mutations/amplifications that might correspond to improved treatment.

Significance: Genetic analysis of treated NSCLC patients will help us find biomarkers in order to better predict patients who will benefit from anti-EGFR-treatment.

P06.063 The analysis of mutations in the EGFR and K-ras genes using LNA-clamp PCR and hybridization with biochips

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The epidermal growth factor receptor (EGFR), a receptor tyrosine kinase whose activation leads to aberrant signaling and cell proliferation in lung adenocarcinomas and colorectal cancers, is a main subject of

new targeted therapies, such as anti- EGFR monoclonal antibodies (cetuximab and panitumumab) or tyrosine kinase inhibitors (gefitinib and erlotinib). Somatic mutations in the *EGFR* gene and *K-ras* gene, coding the downstream GTPase, are associated with the sensitivity to these drugs.

A biochip has been developed for the analysis of mutations in codons 12 and 13 of the *K-ras* oncogene, mutations in codons 858, 719, 790 and deletion in exon 19 of the *EGFR* gene. An approach represents a combination of two-round multiplex PCR and hybridization with immobilized oligonucleotide probes complementary to wild-type and mutated sequences. For specific amplification of mutant fragments in a large excess of wild-type DNA the blocking (clamping) of wildtype sequences with a synthetic nucleotide analogue, locked nucleic acid (LNA) was used. The amplified fragments were labeled via incorporation of fluorescently labeled triphosphate during the second round of PCR. To prove the feasibility of the method the clinical samples from patients with pancreatic cancer, lung adenocarcinoma and colorectal cancer were analyzed.

We consider the biochip-based approach with LNA-clamp PCR as a useful tool for the screening of mutations in the *K-ras* and *EGFR* genes to identify patients who will have a response to anti-EGFR targeted therapies.

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P06.064 Circulating free RNA in NSCLC - a promise for clinical application

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Background: The evidence of circulating nucleic acids in cancer patients is of paramount clinical importance because it provides chances for early diagnosis and better outcomes.

Aim: The aim of this study was to explore the clinical utility of *EGFR* and *hTERT* mRNA expression as markers for diagnosis of NSCLC.

Methods: RNA was isolated with Trizol from 3ml plasma of 45 NSCLC patients and 40 chronic obstructive pulmonary disease (COPD) patients. A preamplification reaction with gene specific primers preceded the qPCR. TaqMan gene expression Assays for *EGFR* (Hs 00193306_m1) and *hTERT* (Hs 00972650_m1) were used. β -actin (Hs 9999903_m1) served as endogenous control. A TaqMan MGB probe (FAM) was used.

Results: The gene expression level of each gene was calculated and given as a relative quantity - RQ. *EGFR* expression was found in all lung cancer patients - mean RQ = 29.39. *hTERT* mRNA was detected in 88% of the patients - mean RQ=17.31. Only 50% of the high risk patients were positive for *EGFR* - mean RQ = 2.09. *hTERT* mRNA was detected in 17 (42.5%) patients of the high risk COPD group - mean RQ=1.02. A statistically significant difference in *EGFR* and *hTERT* mRNA expression could be observed between the two groups of patients - $p=0.0001$.

Conclusion: *EGFR* and *hTERT* mRNA are potential markers for NSCLC diagnosis, whose clinical significance should be replicated in a larger cohort of patients. The preamplification reaction with gene specific primers enhances the sensitivity and detection of free circulating RNAs in plasma of cancer patients.

P06.065 Association between the UGT1A1 polymorphism, dyslipidemia and endometrial cancer risk

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A close association exists between endometrial cancer and dyslipidemia, in our previous study. Uridine diphosphate-glucuronosyltransferases (UGTs) are a family of phase II-metabolizing enzymes involved in glucuronic acid conjugation of sex steroid hormones. UGT1A1 encodes the critically important bilirubin UGT and involved in the conjugation and elimination of estrogens. Bilirubin is an antioxidant that suppresses lipid oxidation and retards atherosclerosis formation. An inverse association between serum bilirubin and coronary heart disease has been reported. Endometrial cancer is the most common gynecologic neoplasm in Western countries and has been increasing over the past several decades in Japan. One of the risk factor of endometrial caner

is the exposure to endogenous and exogenous estrogen throughout life. We hypothesized that UGT1A1 variants may be associated with bilirubin levels and risk of dyslipidemia in endometrial cancer. We studied 312 cases of endometrial cancer, 202 cases had dyslipidemia and 110 cases had nomal value of the lipid, with no history of congenital or acquired liver dysfunction. The assays for genotyping the polymorphisms in the UGT1A1 gene were based on either Invader® assay or direct sequencing. We conducted a case-control study to investigate the associations between UGT1A1 polymorphisms and risk of dyslipidemia in endometrial cancer. Polymorphism of UGT1A1*28 and *60 carriers with higher serum bilirubin concentrations exhibit association with lower risk of dyslipidemia. There is a possibility that the UGT1A1 polymorphism involved in individual variation of conjugation and elimination of estrogens to difference of serum bilirubin levels.

P06.066 Detailed analysis of the independent tumor suppressor loci telomeric to *Tp53* suggests *Skip* and *Myo1c* as novel tumor suppressor gene candidates in this region

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Several lines of evidence indicate an independent, commonly deleted region distal to the *TP53* locus with a potential tumor suppressor activity in a variety of human tumor types. In a recent study, we reported a comparable finding in a rat tumor model for endometrial carcinoma. Using FISH data, we developed a deletion map and narrowed the candidate region to 700 kb, harboring 19 genes. In the present work, we adopted a candidate gene approach to characterize this candidate region further using a set of well-characterized experimental endometrial carcinomas (ECs). We performed real-time qPCR analysis of the 19 genes in a panel of EC tumor and control samples and identified *Hic1*, *Skip* and *Myo1c* as the best candidates. No mutation in coding sequences of *Hic1*, *Skip* and *Myo1c* was detected, and thus low expression levels suggested a haploinsufficient mode of function for these potential tumor suppressor genes. Both *Skip* and *Myo1c* were significantly down regulated at mRNA and/or protein levels, which could be rescued in gene expression restoration assays. This could not be shown for *Hic1*. There is no earlier report on the potential tumor suppressor activity of *Skip* and *Myo1c*. However, they both show an overlapping role in PI 3-kinase/Akt signaling, which is vital for the growth and survival of cancer cells. Moreover, there are reports on tumor suppressor activity of other members of the gene families that *Skip* and *Myo1c* belong to. Functional significance of these two genes in cancerogenesis remains to be investigated.

P06.067 Hereditary mosaic gene inactivation by promoter methylation in colorectal cancer susceptibility

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We have described 3' end deletions of the *EPCAM* gene as a novel cause of Lynch syndrome (also known as Hereditary Non-Polyposis Colorectal Cancer). *EPCAM* is located directly upstream of *MSH2*, a well known mismatch repair gene that is frequently mutated in Lynch syndrome patients. Deletions of the 3' exons of *EPCAM* lead to transcriptional read-through across the *MSH2* promoter, thereby inducing allele specific methylation and inactivation of *MSH2* in tissues expressing *EPCAM*. Thus a mosaic pattern of epigenetic inactivation of *MSH2* is established, that can be inherited over generations. Together with several international partners, we have thus far identified 43 independent families with a 3' end deletion of *EPCAM*, which leaves the promoter region of *MSH2* intact. In these families 18 different deletions have been characterized, all including the two most 3' exons containing the transcription termination signal of *EPCAM*. All deletions appear to have arisen by Alu-repeat mediated recombination events. Methylation of the *MSH2* promoter has been confirmed in mutation carriers from 15 families for which tissue was available. These results show that deletions of the 3' end of *EPCAM* are a recurrent cause of Lynch syndrome. Recently, we obtained additional evidence for an epigenetic

silencing scenario caused by transcriptional read-through of another gene involved in colorectal cancer, suggesting that this phenomenon may be more prevalent than previously thought.

P06.068 Loss of EPS8 expression in a subset of colorectal carcinoma and adenoma

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EPS8 has a conserved actin barbed-end capping function that is required for proper apical morphogenesis and maturation in the intestinal cells. Recent data showed that *EPS8* was overexpressed in advanced stage colon cancers. Our expression microarray analysis of colon cancer cell lines confirmed this overexpression but there were strikingly low levels of *EPS8* in RKO. In our genome wide LOH analysis of colon tumors, we observed considerable LOH at the *EPS8* gene locus. Immunohistochemistry demonstrated that *EPS8* is constitutively expressed at normal colonic mucosa with a dot-like supranuclear cytoplasmic localization. The staining was stronger towards the luminal surface suggesting that *EPS8* plays a role in epithelial differentiation. Compared to matching normal mucosa, 19 colon tumors (4 adenoma, 15 carcinoma) out of 51 (37%) showed strikingly tumor specific *EPS8* protein loss. Of the remaining tumors, 5/51 (2 adenoma, and 3 carcinoma, 10%) showed marked overexpression, while 27/51 tumors (53%) showed retained expression. Mutation analysis revealed a missense mutation (c.794C>T, p.R265C) in exon 8 in RKO. *EPS8* promoter was also methylated in RKO but there was no significant methylation in other cell lines or carcinoma specimens studied. The clear loss of *EPS8* expression in the presence of few genomic alterations in adenomas and carcinomas suggests that down regulation of this gene might be a significant event in the early stages of colorectal carcinogenesis.

P06.069 Polymorphism of estrogen receptor- α gene codon 10 (T392C) in Iranian women with breast cancer

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Breast cancer is one of the most frequent malignancies among Iranian women. Estrogen receptor- α gene (*ESR1*) polymorphism has been found to be associated with breast cancer and clinical features of the disease in Caucasians and Asians. A case study was conducted to establish a database of *ESR1* polymorphisms in Iranian population in order to compare Western and Iranian (Middle East) distributions and to evaluate *ESR1* polymorphism as an indicator of clinical outcome. The *ESR1* gene was scanned in Iranian patients newly diagnosed invasive breast tumors, (150 patients) and in healthy individuals (147 healthy control individuals). PCR single-strand conformation polymorphism methodology and direct sequencing were performed.

The silent single nucleotide polymorphism (SNPs) was performed, as reported previously in other studies, but at significantly different frequencies, with further increasing predictive accuracy in Iranian population.

Data suggest that *ESR1* polymorphisms are correlated with various aspects of breast cancer in Iranian *ESR1* genotype, as determined during pre-surgical evaluation, might represent a surrogate marker for predicting breast cancer.

P06.070 Rapid detection of APC mutations in Slovak FAP families by high resolution melting analysis and protein truncation test

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Familial adenomatous polyposis (FAP) is an autosomal dominant predisposition to colorectal cancer and is caused by germline mutations in the adenomatous polyposis coli gene (*APC*). Classical FAP is characterised by the occurrence of hundreds to thousands of colorectal adenomatous polyps and by several extracolonic manifestations. An attenuated form of polyposis (AFAP) is associated with less than 100 adenomas and later onset of the disease.

In this study we analyzed the *APC* gene for germline mutations in 68 probands from unrelated FAP families of Slovak origin. The mutation analysis of the complete coding region and exon-intron boundaries of the *APC* gene was performed using a combination of high resolution melting analysis and protein truncation test. All positive findings ob-

tained by both screening methods were verified by sequencing analysis.

We identified 22 different germline *APC* mutations and 8 of these were novel. Of the identified mutations, 10 were 1 to 5 bp deletions, 2 were 1 bp insertion and 10 were nonsense mutations, 19 leading to the formation of premature stop codon. Three mutations were in the splice-site region of the *APC* gene. Mutation screening for large genomic alterations in the *APC* gene was performed by multiplex ligation-dependent probe amplification (MLPA). Large *APC* deletions were detected in 3 patients.

The total mutation detection rate was 80% in patients with classical FAP and 9% in AFAP patients. The results of the mutation analysis are important for the preparation of individual treatment strategies for FAP patients.

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P06.071 Diagnosis of Fanconi Anaemia (FA) in dizygotic twins

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Background: FA is a rare autosomal instability syndrome characterized by progressive bone marrow failure, various congenital malformations, predisposition to malignancy and cellular hypersensitivity to cross linking agents. We report on a case of FA in dizygotic twins with characteristic congenital abnormalities and the same deletions of the *FANCA* gene.

Patients & Methods : A twin boy and a girl, aged 6 years old, were referred to the Department of Medical Genetics for genetic investigation after the clinical suspicion of FA. Peripheral blood samples were analysed with cytogenetic and molecular techniques. Matched for age and sex donors were used as controls.

For clastogen-induced chromosome damage both MMC and DEB were added and a minimum of 150 metaphases per case were analysed. A sample was considered as FA positive if the percentage of breaks and radial formations was 7-10 times higher as compared to the control. Molecular investigation was performed using the Multiplex Ligation-dependent Probe Amplification (MLPA) technique to detect deletions of the *FANCA* gene which account for more than 65% of Fanconi Anaemia cases.

Results : Induced breaks and radial formations were detected in both patients. MLPA identified the same deletions involving exons 1-5 and 7-17 of the *FANCA* gene in both patients. Parental molecular testing revealed that the mother was heterozygous for deletions of exons 1-5 and the father for deletions of exons 7-17.

Conclusion : Although the siblings were dizygotic twins, both were compound heterozygous for the same deletions of the *FANCA* gene.

P06.072 Detection of complex mutations in Swedish FAP families

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Familial adenomatous polyposis (FAP), is caused by mutations in the *APC* gene. In Swedish FAP families, 61 different mutations in 96 families analyzed have been found and 27 of those are novel (Kanter-Smoler et al 2008). In classical FAP almost all mutations have been found, whereas in attenuated FAP (AFAP) or atypical FAP 70-80% of the mutations still remain unknown. The main mutation detection methods that have been used are, Sanger sequencing, dHPLC, SSCP and MLPA.

The aims of this study were therefore to investigate the mutation negative AFAP cases and the more complex *APC* gene inactivations resulting in a lower expression of the *APC* gene. In addition mutations affecting splicing and patients with mutations that demonstrate a variation in the penetrance of the disease were also studied.

The methods that have been used include copy number- and expression analysis using the 6.0 SNP arrays and Exon arrays 1.0 from Affymetrix. RT PCR was used for extensive RNA investigations of the *APC* transcripts.

Larger deletions of the *APC* gene including the promoter region and in some cases several other upstream genes have been detected. RNA

studies have also revealed the existence of several splice variants both in mutation carriers and normal controls. In other cases common mutations with lower penetrance among family members have been found. These mutations were located in sites that could interfere with RNA-binding proteins involved in splicing, however modifying genes and other complex mutational mechanisms were not excluded.

P06.073 MutS β exceeds MutS α in dinucleotide loop repair

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The target substrates of DNA mismatch recognising factors MutS α (MSH2+MSH6) and MutS β (MSH2+MSH3) have already been widely researched. However, the extent of their functional redundancy and clinical substance remains unclear. Mismatch repair (MMR)-deficient tumours are strongly associated with microsatellite instability (MSI) and the degree and type of MSI seem to be dependent on the MMR gene affected, and is linked to its substrate specificities. Deficiency in MSH2 and MSH6 is associated with both mononucleotide and dinucleotide repeat instability. Although no pathogenic MSH3 mutations have been reported, its deficiency is also suggested to cause low dinucleotide repeat instability. Germline mutations in MMR genes are associated with hereditary nonpolyposis colorectal cancer (HNPCC). To assess the substrate specificities and functionality of MutS α and MutS β we performed an *in vitro* MMR assay using three different substrate constructs, GT mismatch, 1 and 2 nucleotide insertion/deletion loops (IDLs) in three different cell lines. Our results show that although MutS α alone seems to be responsible for GT and IDL1 repair, MutS α and MutS β indeed have functional redundancy in IDL2 repair and in contrast with earlier studies, MutS β seems to exceed MutS α . The finding is clinically relevant because the strong role of MutS β in IDL2 repair indicates MSH3 deficiency in tumours with low dinucleotide and no mononucleotide repeat instability.

P06.074 Gain of chromosome 2p, recurrent chromosomal aberration in B-CLL

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Chromosomal aberrations are independent prognostic factors in B-cell chronic lymphocytic leukemia (B-CLL).

By improving the cultivation technique using CpG-oligonucleotides and IL-2 stimulation other chromosomal aberrations were identified, especially unbalanced translocations and complex karyotypes. These findings can modify prognosis of patients evaluated by fluorescence *in situ* hybridization (FISH). Recently there was found the other recurrent aberration- gain of chromosome 2p.

Metaphase cytogenetic and molecular-cytogenetic analyses detected gain of 2p in 17 of 225 patients.

We performed metaphase cytogenetic analysis using cultivation technique with CpG-oligonucleotides and IL-2 stimulation. Interphase FISH was used for detection deletions of 13q, 11q, 17p, trisomy 12 and t(14q32). Gains of 2p were confirmed by multicolour FISH and range of gain was determined by multicolour banding.

Gain of 2p was detected in 17 (7,6%) cases. In 13 cases, gain of 2p was detected as unbalanced translocation with the other chromosome. In 3 cases direct 2p duplication was presented, one case had direct 2p duplication together with unbalanced translocation and isochromosome 2p. FISH identified chromosomal aberrations in 16 cases; 7 cases had 13q deletion as a sole aberration, 6 cases had 13q and 11q deletions, one case had 11q deletion and +12, one case had 17p deletion. Correlations between gain of chromosome 2p and some clinical/biological parameters will be presented.

The cultivation technique using CpG-oligonucleotides and IL-2 stimulation is an efficient method that can improve quality of cytogenetic analysis. Detection of the other aberrations i.e. gain of chromosome 2p can provide new prognostic information in B-CLL, which is presented.

P06.075 Interleukin 1B gene (IL-1B) and interleukin 1 receptor antagonist gene (IL-1RN) polymorphisms and the risk of gastric cancer

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Gastric cancer is one of the leading causes of cancer death worldwide and remains a significant global health problem, with widely varying geographical distribution. Association between gastric cancer risk and polymorphisms in the genes coding for cytokines was reported in case-control studies. The aim of this study was to investigate the polymorphisms of IL1B Ex5+14C>T (rs 1143634) and IL1RN Ex5 -35T>C (rs 419598) in a Romanian population. A total of 55 gastric cancer patients and 105 healthy controls were included. Genomic DNA was extracted from patients and controls blood using Wizard Genomic DNA Purification Kit (Promega). The polymorphisms were genotyped using Taq Man SNP Genotyping Assays (Applied Biosystems) and the different alleles were discriminated according to the fluorescence intensity of Fam and Vic (RotorGene 6200 HRM-Corbett). To investigate the association of these polymorphisms with gastric cancer we used χ^2 test. Allelic distributions were examined for deviation from their corresponding Hardy-Weinberg equilibrium. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated to assess the relative risk conferred by a particular allele and genotype. The results indicate that allele IL1RN Ex5 -35C could increase the genetic susceptibility of gastric cancer (OR 3.22; 95% CI 1.08-9.60). The polymorphism of IL1B Ex5+14C>T is not associated with gastric cancer risk (OR 0.83; 95% CI 0.24-2.85). To our knowledge this is the first study of these polymorphisms in an Eastern European population and further larger studies of genetic variation in IL1-B and IL-1RN are required.

P06.076 Comparison of the repair kinetics of the UVA- and UVB-induced cyclobutane pyrimidine dimers in the genome of human keratinocytes and its relation to expression of DNA repair genes

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Solar ultraviolet radiation (UVR) by inducing DNA photo-lesions, has become the prime cause of most skin cancers. These cancers could be prevented if we protect ourselves from UVR.

UVB is directly absorbed by DNA and induces different forms of lesions like cyclobutane pyrimidine dimers (CPDs). In contrast with UVB, UVA is indirectly absorbed by DNA. It has long been considered that the UVA component of solar UVR carries a minimal risk for skin carcinogenesis, as it does not lead to the formation of CPD, not least because DNA does not absorb UVA. Most sunscreens filter out UVB absorption, but they cannot block most of UVA, so they do not help to prevent skin cancer. A better assessment of the routes by which UVA and UVB induce CPDs, may lead to prevention of skin cancer.

This study focuses on the differential expression levels of genes coding for components of the nucleotide excision repair pathway and also viability of the human keratinocytes (HaCaT cell line), before and after irradiation with UVA and UVB.

P06.077

Towards an understanding of genetic selection in breast cancer

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The cancer genome contains frequent chromosomal abnormalities and gene mutations. Nevertheless, the cell must maintain the capability of self-renewal, unlimited growth potential, and the ability to form tumours *de novo*. We are examining selective forces in the breast cancer genome, to determine if the minimal essential genome encodes proteins that are consistently targets of therapeutic drugs. Analysis of the genomes of 243 previously reported breast tumours (*Gen Res.* 2006; 16:1465) revealed 766 unstable (amplified and/or deleted) and 815 stable (or quiescent) contiguous genomic regions. The stable regions had 10,232 protein coding genes of which expression of 8,954 of these genes were measured in the Affymetrix HG U133 Plus 2.0 array. Using datasets reporting differential gene expression in primary breast cancer samples vs. matched normal controls (*BMC Can.* 2007; 7:55), we identified that 70% (6247/8954) of the genes had both stable copy number and stable expression. The genes with stable expression were characterized by bioinformatic analyses of gene on-

tology and biological pathways. We determined that the breast cancer genome preferentially preserves functions that include cellular metabolic processes, regulation of gene expression, DNA packaging (chromatin and nucleosome assembly), cellular component assembly, RNA metabolic process and regulation of apoptosis ($p \leq 0.01$). Conservation of this minimal gene set may explain the effectiveness of certain chemotherapeutic agents (doxorubicin, cisplatin, carboplatin, tamoxifen, estradiol, fluorouracil, etc) due to their actions on multiple gene products in this set.

P06.078 Study of 20 glioblastoma patients treated in Kaunas University of Medicine during 2003-2006y

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Glioblastoma multiforme (astrocytoma grade IV, WHO) is the most common and most aggressive primary brain tumor in adults. Supposedly, glioblastoma arises from altered glia cells or/and brain stem cells. These tumors are characterized by intratumoral heterogeneity, invasive growth nature and rapid recurrence. Despite aggressive multimodal therapy, prognosis for glioblastoma patients still remains poor, as most patients die within the first 12-15 months after diagnosis. This situation has not significantly changed in the last 20 years. Here, we report 20 glioblastoma patient cases followed up in the Department of Neurosurgery, Kaunas University of Medicine during 2003-2006y. Patient clinical data - age, sex, tumor localization, treatment, relapse free survival (RFS) and overall survival (OS) were estimated (see Table). The chromosome 9p21 and its CDKN2A locus, with the p16(INK4A) and p14(ARF) genes are recognized as the most frequent genomic regions involved in the pathogenesis of glioblastoma were investigated.

Results: there were no significant points for relapse free survival and overall survival, we think because of small glioblastoma patients group.

| Patient characteristics | | Relapse Significance, p | Survival Significance, p |
|-------------------------|----------------|---------------------------|----------------------------|
| Sex | 9 F 11 M | 0.175 | 0.133 |
| Age, y | 48.7 (SD 11.3) | 0.125 | 0.201 |
| Resection | 20/20 | N/A | N/A |
| Radiation therapy | 20/20 | N/A | N/A |
| Chemotherapy (Temodal) | 15/20 | 0.875 | 0.282 |
| p14 deletion | 4/17 (23.5%) | 0.04 | 0.456 |
| p16 deletion | 3/17 (17.6%) | 0.130 | 0.763 |

P06.079 Risk Assessment and BRCA1/BRCA2 testing results of Hereditary Breast Cancer Patients in Turkey

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Introduction and objectives: Breast cancer, with an average frequency of 10-12% is a common health concern for all ethnic groups around the world. Known risk factors vary from sex/age to environmental factors. The inheritance of cancer susceptibility genes BRCA1 and BRCA2 are risk factors for familial breast cancer development. About 85-90% of breast cancer cases are sporadic and only the remaining 5-10% account for early onset familial cancer. Individuals that carry BRCA1 and/or BRCA2 gene mutations, life time risk of developing breast cancer significantly increases.

Here we report novel mutations of BRCA1 and BRCA2 genes identified in Turkish families with positive family history and risk estimation for these cases.

Methods: This study presents findings from families that have been referred to our institutions for breast cancer risk estimation and genetic testing. The initial step with all cases is genetic counseling to obtain family history, explain the benefits and risks of genetic testing, probable impact of results on the family members, and the limitations of genetic testing. Those cases that agree for the testing to proceed an

informed consent was obtained.

Results: Analysis of twenty-five cases revealed novel mutations on BRCA1 and BRCA2 genes respectively

Conclusions: It is known that the frequency of breast cancer differs with ethnic origin. Assessment of cancer risk and probable life time cancer risk development takes into account the mutations found in the BRCA genes. Knowing mutations unique to ethnic origins is of interest for improving successful risk calculation.

P06.080 Splicing Functional Assays of DNA variants of the BRCA1 and BRCA2 genes.

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The analysis of the deleterious effect of genetic variants in disease genes is usually focused on the predicted effect on protein function but an increasing amount of evidence indicates that there can be deleterious effects through the disruption of the splicing process.

We have investigated the presence of aberrant splicing of *BRCA1/2* in hereditary breast/ovarian cancer (HBOC). About 15% of individuals undergoing genetic testing carry a DNA variant of unknown physiological significance that hamper genetic counselling in HBOC.

We examined the functional consequences on splicing of 24 *BRCA* variants detected in 688 breast/ovarian cancer families that were bioinformatically preselected with splicing specific tools: NNSPlice (splice site analysis), ESEfinder and ESRsearch (detection of splicing enhancers and silencers). They were assayed by RT-PCR of lymphocyte mRNA and/or hybrid minigene experiments in HeLa and nontumor breast epithelial cells. Another 33 putative splicing variants from the BIC mutation database were generated by site-directed mutagenesis and analyzed by hybrid minigenes assays.

Twenty-eight variants altered the splicing process by different mechanisms, including disruptions of the canonical splice sites, the polypyrimidine tract and splicing enhancers/silencers, and creation of cryptic splice sites. We found that any type of variant (missense, synonymous, nonsense, insertions/deletions) can be associated with defective splicing.

Furthermore, twenty mutations were predicted to truncate the *BRCA* proteins and/or to delete essential domains, suggesting that aberrant splicing of *BRCA1/2* may represent an important etiopathogenic mechanism in HBOC.

The identification of splicing disruptions by functional assays is a valuable tool to discriminate between benign polymorphisms and pathogenic mutations.

P06.081 Tumour suppressor function of both of the BRCA1 and BRCA2 genes has a low threshold for splice site mutation.

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The majority of inactivating mutations in the *BRCA1/BRCA2* tumour suppressor genes are either small frame-shifting in/dels or nonsense mutations obviously causing protein-truncating events associated with increased risk of breast and/or ovarian cancer, (BIC database1). The classification of non-truncating sequence variations of unknown significance (VUS) is more difficult. Most of these VUS are comprised of synonymous or non-synonymous coding SNP's, intronic and/or 5' and 3' sequence variants potentially affecting normal gene expression by variously interfering with normal promotor function, mRNA stability and/or normal mRNA splicing. Easton et al² have attempted to better characterize the clinical significance of these VUS by: assessment of family cancer history, co-occurrence in trans with a known deleterious mutation, and/or co-segregation with disease in HBOC families. To further analyze this data an information theory-based splicing mutation analysis was used to predict the amount of full-length mRNA produced by these variants. A significant proportion of the 43/1433 VUS classified as clinically significant by Easton et al. (12/26 in *BRCA1* and 8/17 in *BRCA2*) were predicted to disrupt normal splice function, though 3 of these variants were also non-synonymous coding SNP's. 50% of these damaging splice variants (6/12 in *BRCA1* and 4/8 in *BRCA2*) predict only partial loss of splice site recognition. This high prevalence of leaky splicing mutations in *BRCA1/BRCA2* genes is consistent with

the possibility that high levels of these genes products are essential for tumour suppression, and alternative splicing at these loci may be diverting full length mRNA to inactive isoforms.

1. <http://research.ncbi.nlm.nih.gov/bic/>

2. Easton et.al 2007 AJHG:81:873_883

P06.082 Assessing pathogenicity of unclassified variants in hereditary colorectal cancer susceptibility genes

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Genetic testing of susceptibility genes in hereditary colorectal cancer (CCR) is applied in clinical practice to predict risk of developing CRC. Many of the mutations identified result in premature termination of translation and thus in loss of function of the encoded proteins. These variants are considered pathogenic mutations. However, a substantial proportion of the variants found are nucleotide substitutions, either within the coding sequence (missense or silent variants) or in introns, which do not lead to such a premature termination of the translation. The question of whether these variants, referred to as unclassified variants (UVs), affect the normal function of encoded proteins and therefore contribute to the development of disease is essential in genetic testing. Characterizing the significance of such variants represents a major challenge for medical management of patients in whom they are identified.

The aim of this study was to assess the pathogenicity of 38 exonic and 13 intronic UVs found in *APC*, *MYH* and *MMR* genes in families with hereditary colorectal cancer. A combination of approaches was followed that included: segregation with the disease within families, absence in control individuals, co-occurrence with known deleterious mutations, effect on mRNA for putative splice-site mutations, effect on protein function by bioinformatic analysis, and specifically for *MMR* variants, tumour microsatellite instability status and DNA mismatch repair protein expression by immunohistochemistry.

P06.083 The case report of Hereditary Diffuse Gastric Cancer with causal mutation in *CDH1* gene

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Hereditary diffuse gastric cancer (HDGC) is the autosomal dominant susceptibility for diffuse gastric cancer, a poorly differentiated adenocarcinoma that infiltrates into the stomach wall causing thickening of the wall (*linitis plastica*) without forming a distinct mass (ring carcinoma). *CDH1*, encoding the protein E-cadherin, is the only gene known to be associated with HDGC. Mutations in *CDH1* account for approximately one-third of HDGC. The majority of the cancers in individuals with *CDH1* mutations occur before the age of 40 years. *CDH1* mutations are also correlated with breast, colorectal, thyroid and ovarian cancer.

The E-cadherin is cell-cell adhesion molecule whose reduced expression or loss of function is regarded as one of the main molecular events involved in dysfunction of the cell-cell adhesion system, triggering cancer proliferation, invasion and metastasis.

In this work we demonstrate a case report of molecular analysis of *CDH1* gene, exemplified demonstrating anticipation of HDGC. Proband is 27-year-old woman whose sister died at the age of 30 to gastric cancer, her mother died at the age of 47 to pancreatic cancer and her grandmother died at the age of 63 to gastric cancer. We found the causal mutation IVS15-6C> G in the *CDH1* gene of proband. The uncle of proband (mother's brother 57-year-old) was negative for this causal mutation. Thanks to performed molecular analysis the proband may undergo life-saving total gastrectomy.

P06.084 qPCR-HRM combining to unlabeled probes : a single technique used for point mutation prescreening, large gene rearrangement detection and genotyping of frequent SNPs in *BRCA1* and *BRCA2* genes

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Quantitative PCR High Resolution melting (qPCR-HRM) allows in one step the detection of point mutation and gene rearrangements. Due to recurrent SNPs in *BRCA1* and *BRCA2* genes, a large amount of sequencing work remains after qPCR-HRM, as melting curves for SNPs and deleterious mutations may be similar. To reduce sequencing, we developed genotyping of five recurrent SNPs in *BRCA1* and *BRCA2* genes by unlabeled probes and melting curves analysis. Probes are 30 to 35 bp 3' phosphorylated oligonucleotides corresponding to the mutated allele of each SNPs and included in PCR mixes. Asymmetric PCRs (1:10 primers ratio) were performed with fluorescent saturating dye (Resolight dye®) on LightCycler® 480. Genotyping is accomplished by monitoring the post-PCR melting of probe-target duplexes. Sensitivity and specificity of the assays were evaluated with a blind screening of 92 samples previously sequenced. All the heterozygous and homozygous were correctly genotyped by unlabeled probes and melt curve analysis. In seven cases, sequence alterations were distinguished from SNPs. For example, previous gene scanning analysis showed similar melting curves for the c.2415_2416del mutation and the c.2311C>T SNP of the *BRCA1* gene. The second example is the distinction by unlabeled probes between the c.1-11C>T variant and the c.1-26G>A in the *BRCA2* gene.

qPCR-HRM combined with unlabeled probes is a simple, sensitive and rapid method which allows detection of point mutation and of gene rearrangements and genotyping of SNPs. This method could be extended to other recurrent SNPs in *BRCA1* and *BRCA2* genes.

P06.085 Hereditary leiomyomatosis and renal cell cancer: A Nationwide study of all families referred for *Fumarate Hydratase* germline mutation analysis.

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¹VU University Medical Centre, Amsterdam, Netherlands, ²Radboud University Medical Centre, Nijmegen, Netherlands, ³Maastricht University Medical Centre, Maastricht, Netherlands, ⁴Leiden University Medical Centre, Leiden, Netherlands, ⁵Erasmus Medical Centre, Rotterdam, Netherlands, ⁶University Medical Centre Groningen, Groningen, Netherlands, ⁷Academic Medical Centre, Amsterdam, Netherlands, ⁸University Medical Centre Utrecht, Utrecht, Netherlands, ⁹Royal Marsden NHS Foundation Trust, London, United Kingdom. **Background** Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an autosomal dominant syndrome characterised by multiple cutaneous piloleiomyomas, uterine leiomyomas and papillary type 2 renal cancer. HLRCC is caused by germline mutations in the *fumarate hydratase* (*FH*) gene. Clinical expression is highly variable. We evaluated the yield of *FH* germline mutation analysis in a cohort of suspected HLRCC kindreds, assessed clinical variability among families with a demonstrated mutation, and propose diagnostic criteria and indications for *FH* mutation testing.

Methods Clinical data were collected from all families referred for *FH* mutation analysis in the Netherlands. An MLPA test was performed in families with negative *FH* sequence analysis.

Findings In 14 out of 33 families we identified 11 different pathogenic *FH* germline mutations, including four novel mutations and one whole-gene deletion. Clinical data were available from 34 *FH* mutation carriers. Cutaneous leiomyomas were present in all *FH* mutation carriers above 40 years. 10 out of 20 female *FH* mutation carriers underwent surgical treatment for symptomatic uterine leiomyomas at 34 years on average. Two *FH* mutation carriers had kidney cancer; the histological subtypes were papillary type 2 and Wilms' tumour, respectively.

Interpretation All *FH* mutation positive families harboured patients with cutaneous leiomyomas, contrasting with none of the families negative for *FH* mutations. An MLPA test should be part of *FH* mutation testing. *FH* mutation carriers frequently had early-onset, severely symptomatic uterine leiomyomas, whereas renal cancer was infrequent. Multiple cutaneous leiomyomas may be diagnostic for HLRCC.

P06.086 Molecular diagnostics strategy to identify hereditary non-polyposis colorectal cancer patients

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Introduction. Hereditary non-polyposis colorectal cancer (HNPCC), also called Lynch syndrome, is an autosomal dominantly inherited syndrome predisposing to the early development of cancers of colon, rectum, endometrial, small bowel and urinary tract and accounts for ~5% of all colon cancer cases. Molecular investigations have shown that most HNPCC families are associated with constitutional mutations in a class of genes (called *MLH1*, *MSH2*, *MSH6*, *PMS2* and probably others) involved in DNA mismatch repair. *MLH1* and *MSH2* genes account for approximately 90% of HNPCC families identified germline mutations. Most of these mutations are point mutations and small insertions or deletions. The large genomic deletions explain a significant proportion and epimutations in *MLH1* a smaller fraction of point mutation-negative cases with MMR protein loss in tumor tissue. The aim of the study is to improve the diagnostic strategy of Lynch syndrome and characterize the MMR gene mutations of Estonian HNPCC families.

Methods. A systematic analysis of patients from Estonian HNPCC-suspected families is performed using complex analysis by immunohistochemistry, microsatellite instability, and by detection of gene mutation and changes in methylation profiles.

Results. A comprehensive analysis of samples from 75 HNPCC-suspected patients is ongoing, we will present preliminary results.

Conclusion. Based on traditional molecular genetics and combined with epigenetics, multiple detection methods can accurately diagnose HNPCC. As the results of the study, the precise molecular diagnostic scheme for diagnosing of HNPCC will be worked out for clinical use in Estonia.

P06.087 Promoter region methylation status of *MGMT* and *THBS1* genes in breast carcinoma patients

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Carcinogenesis is a multistep process with genetic and epigenetic changes playing role from the very beginning to the metastatic level. Breast cancer is a major health problem among women all over the world. Changes in methylation patterns of a number of genes associated with cell growth have been reported in breast cancer. In the current study, we studied methylation status of *MGMT* and *THBS1* genes in breast carcinoma patients. Paraffin embedded tissue samples from 47 invasive breast carcinoma patients were included in the study. Promoter regions of *MGMT* and *THBS1* genes were amplified by methylation specific polymerase chain reaction following bisulphite modification. We detected methylation of *MGMT* gene in 4 patients (8.51%) whereas *THBS1* promoter methylation was observed in one patient (2.12%). Statistical analyses were performed by Pearson's chi square test to investigate whether methylation was related with age, menopausal status, lactation, parity, histological tumor type, local recurrence, axillary metastatic lymph nodes and remote metastasis of the patients. We did not detect a significant relationship between the methylation statuses of the promoters of the two genes with the above parameters. Methylation status of different genes has been reported to be heterogeneous in breast carcinoma, although we did not detect a significant relationship, we believe studies including increased number of samples and genes will provide more information.

P06.088 Somatic mutations in juvenile polyps from *BMPR1A* and *SMAD4* mutation carriers

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Juvenile polyposis syndrome (JPS) is an autosomal dominant disorder that predisposes to gastrointestinal polyps and a cancer risk of 10-50%. Germline mutations in the genes *BMPR1A* or *SMAD4* have been identified in approximately half of JPS patients. These 'first hit' mutations are often point mutations or small deletions but also large chromosomal deletions occur. So far however, little data is available on the nature of the somatic, 'second hit' mutations in polyps of JPS patients. In this study, we have screened for somatic mutations in twenty-four juvenile polyps from three individuals harbouring a pathogenic *BMPR1A* (583C>T; G195X; patients P1 & P2) or *SMAD4* (1244-1247delACAG; patient P3) germline mutation.

We identified consistent allelic loss of the *BMPR1A* wild-type allele at the location of the first hit mutation in 45% (5/11) of juvenile polyps from patients P1 (4/9) & P2 (1/2). Loss of heterozygosity (LOH) at informative STR markers indicated that the entire wild-type *BMPR1A* gene was lost in three of the five polyps. Mutation analysis of epithelial and stromal tissue isolated by laser microdissection from one polyp showed that LOH solely occurred in the epithelium. This was verified by FISH probes covering the *BMPR1A* region which additionally specified the size of the chromosomal alteration.

Immunohistochemical staining of the juvenile polyps from patient P3 revealed the absence of *SMAD4* expression in the epithelial (80%, 12/15) and stromal (60%, 9/15) compartments. However, no second hit mutations were identified in these polyps, despite extensive screening for sequence variations, chromosomal rearrangements, and promoter methylation.

P06.089 Sensitive detection of *KRAS* and *BRAF* mutations using mutant-enriched PCR and reverse-hybridization teststrips

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KRAS and *BRAF* are key players in growth factor receptor induced signalling pathways. Somatic mutations in the two genes are known to play a role in oncogenesis and are found in various types of tumors. *KRAS* and *BRAF* mutations are also known to be predictive for the response to cancer therapy with anti-EGFR antibodies.

We have developed a reverse-hybridization StripAssay targeting ten *KRAS* codon 12/13 mutations as well as *BRAF* V600E. The test is based on PCR in the presence of wild-type suppressors (mutant-enriched PCR), followed by hybridization to teststrips presenting a parallel array of allele-specific oligonucleotide probes. The hybridization and detection steps can be carried out automated using commercially available instrumentation. StripAssay performance was evaluated on genomic DNA obtained from cultured cell lines, formalin-fixed paraffin-embedded tissue and stool.

Using dilutions of DNA from tumor cell lines into normal DNA, each mutation was shown to be detectable at levels as low as 1%. DNA containing various proportions of mutant *KRAS* was analyzed by StripAssay hybridization and compared to results from real-time PCR, dideoxy sequencing and pyrosequencing. Dideoxy sequencing and pyrosequencing failed to detect levels of 12.5% or lower, while StripAssay hybridization and real-time PCR unambiguously identified as low as 1% of mutant *KRAS*.

The existing StripAssay is currently being extended to contain additional mutations, such as *KRAS* codon 61 variants. The simultaneous detectability of multiple mutations in a single experimental set up with excellent sensitivity will make the StripAssay a useful tool for the *KRAS*/*BRAF* mutation assessment on tumor samples.

P06.090 KRAS Variant Detection on the Applied Biosystems 3500xL Genetic Analyzer

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The *KRAS* gene encodes a protein in the RAS-MAPK pathway, one of several signaling cascades downstream of EGFR activation. EGFR signaling pathways are important in the development and progression of several cancers, with *KRAS* gene mutations common in pancreatic, lung, and colorectal cancers. Activating *KRAS* mutations have been described most frequently in codons 12 and 13, these mutations result in constitutively active form of *KRAS* causing EGFR pathway signaling independent of EGFR activation; thus making therapeutic agents

that block EGFR ineffective. Here we describe limit of detection experiments for the detection of sequence variants in codons 12 and 13 of the *KRAS* gene. Methodologies investigated include capillary electrophoresis (CE) DNA sequencing, Shifted Termination Assay (STA) detection, single-base extension, pyrosequencing, real-time PCR, and high resolution melt (HRM). Two sample types were used to assess the limit of detection: 1) mixtures at various percentages of gDNA extracted from *KRAS* mutant and wild-type cancer cell-lines; and 2) mixtures at various percentages of *KRAS* mutant and wild-type cells that were formaldehyde fixed and paraffin embedded to create a cell block from which gDNA was extracted. The analytic sensitivity of the above methodologies is described with a detection of *KRAS* variants in codons 12 and 13 possible at a level of 1%. Further, copy number variation (CNV) assessment of *KRAS* mutant cell line gDNA was performed since it is known that cancer cell lines have CNV of the *KRAS* gene. For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use

P06.091 SNaPshot as a sensitive molecular tool for prognostic determination of K-RAS mutation status in patients with colorectal cancer in different clinical stages

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The *K-RAS* has been the first molecular marker used to predict successful response to specific biological treatment with anti-EGFR monoclonal antibody (MoAb) in the first line treatment of metastatic colorectal cancer (mCRC).

We established a rapid and inexpensive assay based on Multiplex SNaPshot kit (Applied Biosystems) to facilitate the identification of *K-RAS* mutations in codons 12 and 13 on a routine diagnostic basis.

At first, DNA samples isolated from the formalin-fixed paraffin-embedded tissues have been tested for a diagnostic indication of MoAb targeted therapy in patients with mCRC. The *K-RAS* mutations were detected in 182 out of 508 samples (35,7%). Another clinical question we addressed, was the distribution of *K-RAS* mutations in CRC in different clinical stages. Prospective screening included 131 patient samples from fresh resected tumors. Patients were classified according to age, sex, clinical stage, site of tumor, EGFR expression, *p53* and *BRAF* mutation status. We compared these factors in respect to the *K-RAS* mutation status.

Surprisingly, outcomes pointed out a higher mutation rate of *K-RAS* oncogene in clinical non-metastatic stage II(38,8%) in comparison to metastatic stage III(31,3%). Therefore, we intend, that monitoring during the treatment could explain closer the relationship between *K-RAS* status and tumor behavior, tendency to metastasis and treatment response. High mutation rate of *K-RAS* in clinical stage II seems to be important to focus this molecular marker as a crucial prognostic factor in treatment of CRC. Hence, the *K-RAS* status could play a key task in decision to apply adjuvant therapy. Study was supported - MHSR-2006/26-NOU-02.

P06.092 Multiplex RT-PCR assay for rapid and cost efficient screening of fusion gene transcripts in acute leukemia.

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The aim of this study was to validate the application of an "in house" designed multiplex RT-PCR for the detection of 8 most frequent fusion gene transcripts in acute leukemia (E2A-PBX1, TEL-AML1, AML1-ETO, PML-RAR α , MLL-AF4, CBF β -MYH11, BCR-ABL, SIL-TAL) for use in routine molecular diagnostic practice. A total of 78 patient samples at presentation were tested (48 AML and 30 ALL). For evaluation all samples were tested in parallel with HemaVision Full kit (DNA Technology).

A total of 19 cases with fusion genes transcripts were identified (TEL-AML - 1, BCR-ABL - 4, AML-ETO - 2, PML-RAR α - 8, MLL-AF4 - 1, CBF β -MYH11 - 2, SIL-TAL - 1). This allowed risk stratification for 25% of AML cases and 23% of ALL cases. In combination with detection of MLL-AF9 fusion gene transcript, MLL-PTD, FLT3-ITD, FLT3-TKD testing and detection of mutations in NPM1 this method allowed evaluation of prognostic for the most of our AML patients. This method em-

ploys a nested-PCR approach, thus being suitable for MDR monitoring with an almost identical sensitivity as HemaVision assay.

Overall our assay exhibited good sensitivity and specificity and allowed identification of fusion gene transcripts in 24% of acute leukemia. Compared with commercially available kits like HemaVision it is considerably cheaper and for our lot of patients identified the same fusion gene transcripts as HemaVision assay.

This work was supported by the grant PN 41-087 from the Romanian Ministry of Education and Research. The authors express their gratitude to European LeukemiaNet for their permanent support.

P06.093 Li-Fraumeni in the Baltics - it's not so rare, it's not so usual, it's so timely.

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Germline TP53 gene mutations are associated with complex cancer predisposition syndrome, the Li-Fraumeni syndrome (LFS), predisposing to wide spectrum of malignant tumors, especially sarcomas, early-onset breast cancer, brain and adrenocortical carcinomas. Recently, the estimated prevalence of germline TP53 mutations may vary from 1:5000 to 1:20000.

Of note, genetic epidemiology, genotype-phenotype correlation and clinical presentation of LFS are completely unknown in Northern-Eastern Baltic sea region (Lithuania, Latvia and Estonia). Given the strong founder effect in BRCA1 gene in Lithuania, there is a possibility for founder effect in other tumor predisposition genes. In Hematology, oncology and transfusion medicine center of Vilnius university hospital Santariskiu clinics we prefer less stringent modified Chompret criteria (2009) and prescreen TP53 2-11 exons by high resolution melting (HRM) analysis followed by direct automatic sequencing.

During one year period 3 confirmed TP53 carriers families (4 patients) were identified. Here we discuss the peculiarities of the phenotypic expression in these patients and describe the novel germline TP53 mutation. Two of patients conformed to classic LFS criteria, well described p.R282W mutation was revealed in one osteosarcoma patient and p.C199G mutation was found for the first time in germline status (submitted to IARC database) in one breast sarcoma patient. One patient presented as apparently BRCA1-related breast/ovarian patient, which was found negative for BRCA1 mutations but positive for TP53 p.R273C mutation. Additional genetic modifiers (MDM2 SNP307G; T53 R72P, PIN3) were assessed and data will be presented in the context of clinical sense.

P06.094 Alterations of LATS2, SPP1, STK11, VIPR1 and S100A2 are lung cancer type specific

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Molecular heterogeneity within non-small cell lung cancer (NSCLC) is evident in the variable presence of mutations, but molecular differences that may be associated with specific types are either unidentified or controversial. In an attempt to find novel molecular differences between squamous cell carcinoma (SCC) and adenocarcinoma (ADC), we created a DNA microarray platform (MA) by which expression levels of 3060 genes involved in carcinogenesis and apoptosis are monitored. The expression levels of candidate genes that were chosen on the basis of MA results were also validated on RT-PCR, and those genes further investigated for mutations, copy-number changes and methylation. We examined 118 NSCLC tumours of known stages and types, as well as adjacent non-tumour tissue. By means of MA, more than 40 genes, most of them involved in transcription or transcription regulation, were detected as having differential expression in comparison to adjacent healthy tissue. LATS2 and STK11 genes were down-regulated regardless of the type of NSCLC, but further mutation detection showed that alterations are significant only for SCC. In S100A2, SPP1 and VIPR1, we discovered significant differences between ADC and SCC already on expression level. S100A2 and VIPR1 were significantly down-regulated and SPP1 up-regulated in ADC in comparison to SCC. We were able to identify the causes of down-regulation of VIPR1 and LATS2, the former being a copy-number alteration and the latter methylation. Our findings confirm LATS2, STK11 and VIPR1 as tumour suppressors and identify novel changes and genes that clearly show differences between NSCLC histotypes on a molecular level.

P06.095 Women with Lynch Syndrome: perception of cancer risks, follow-up and prophylactic surgery

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Lynch syndrome predisposes to colorectal cancer and also to gynaecological cancers. Screening recommendations for colorectal cancer are well established whereas gynaecological screening is still in debate. We evaluated follow-up, perception of cancer risk and attitude regarding prophylactic gynaecological surgery for 24 women with Lynch Syndrome,

All women had an adequate digestive follow up (21 of 24 had colonoscopies at least every 2 years) but their gynaecological follow up is less systematic (only 14 of 24 patients had ultrasonography and less than 50% also had an endometrial biopsy). Forty seven percents of patients had an accurate perception of their colorectal risk of cancer but only 33 % also had an accurate perception of their endometrial risk of cancer and 8 % for ovarian cancer risk. If gynaecological prophylactic surgery was proposed, they would be 63 % to consider this option but only after menopause. Almost 90% of the patients (21/24) discussed of colorectal cancer risk with their gastroenterologist but only 54% discussed about the gynaecological risk. On the same way, only 50% of these patients discussed about the gynaecological risk with their gynaecologist and 10 women (41%) did not mention their genetic predisposition to their general physician.

Women with Lynch syndrome are less aware of their risks for extra-colonic cancers and undergo endometrial cancer screening less often than colonoscopy. Given the increased risks for endometrial and ovarian cancers, sustained efforts to inform women and physicians of cancer risks, screening recommendations and possibility of prophylactic surgery are important.

P06.096 Characterization of germline mutations of *MLH1* and *MSH2* in suspected South American Lynch syndrome families

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Introduction - Lynch syndrome (LS) is an autosomal dominant syndrome predisposing to the early development of colorectal cancer (CRC), endometrium, ovary, small intestine, stomach and urinary tract. LS is caused by germline mutations in the DNA mismatch repair genes, mostly *MLH1* or *MSH2*, which are responsible for more than 85% of known germline mutations in patients with SL.

Objectives - Search for germline mutations in *MLH1* and *MSH2* genes in 99 Brazilian suspected unrelated LS patients.

Material and Methods - 99 DNAs were obtained from peripheral blood and subjected to PCR followed by direct sequencing in both directions of all exons and intron-exon junctions regions of *MLH1* and *MSH2* genes.

Results - We found 32 *MLH1* or *MSH2* pathogenic mutations, being 2 individuals with 2 mutations. Thirty pathogenic mutations were found, being 21/41 (51.27%) fulfilling the Amsterdam and 9/58 (15.5%) the Bethesda criteria. Thirteen pathogenic mutations were described for the first time. Currently, these findings are available on LOVD database.

Conclusions - The results were consistent with literature. Moreover, higher frequency of *MLH1* or *MSH2* mutations was observed in patients with Amsterdam criteria, confirming their high specificity. The number of new pathogenic mutations in *MLH1* or *MSH2* genes found in this study point for the requirement to screen suspected LS families, mainly regarding the Brazilian families complex genetic background.

P06.097 The risk of urothelial bladder cancer in Lynch syndrome is increased, in particular among *MSH2* mutation carriers

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Background: Colorectal, endometrial and upper urinary tract tumours are characteristic for Lynch syndrome. The aim of this study was to establish whether carriers of mutations in *MSH2*, *MLH1* or *MSH6* are

at risk of urinary bladder cancer.

Methods: Carriers and first-degree relatives of 95 families with a germline mutation in the *MSH2* (n=43), *MLH1* (n=26), or *MSH6* (n=26) gene were systematically questioned about occurrence of carcinoma. The cumulative risk of cancer occurring before the age of 70 years (CR70) was compared to CR70 of the general Dutch population. Microsatellite instability (MSI) testing and/or immunohistochemistry (IHC) was performed on bladder tumours.

Results: Bladder cancer was diagnosed in 21 patients (90% men) from 19 Lynch syndrome families (15 *MSH2*, 2 *MLH1*, and 4 *MSH6*). CR70 for bladder cancer was 7.5% [95% CI 3.1-11.9%] for men and 1.0% [95% CI 0-2.4%] for women, resulting in relative risks for mutation carriers and first-degree relatives of 4.2 [95% CI 2.2-7.2] for men and 2.2 [95% CI 0.3-8.0] for women. Men carrying an *MSH2* mutation and their first-degree relatives were at highest risks: CR70 for bladder and upper urinary tract cancer being 12.3% [95% CI 4.3-20.3%] and 5.9% [95% CI 0.7-11.1%]. Bladder cancer tissue was MSI positive in 6/7 tumours and loss of IHC staining was found in 14/17 tumours, indicating Lynch syndrome aetiology.

Conclusion: Patients with Lynch syndrome carrying an *MSH2* mutation are at increased risk of urinary tract cancer including bladder cancer. In these cases surveillance should be considered.

P06.098 Evaluation of pathogenicity of single-base germline changes involving the mismatch repair genes *MLH1*, *MSH2* and *MSH6* in diagnostics of Lynch syndrome.

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Lynch syndrome is an inherited disease caused by germ-line mutation in mismatch repair genes such as *MLH1*, *MSH2*, and *MSH6*. A proportion of families are characterized by nucleotide substitutions, unclassified variants (UVs) have been found, in order to use the variants for predictive testing in persons at risk, their pathogenicity has to be evaluated.

We examined 7 UVs detected in *MLH1*, *MSH2* or *MSH6* genes in patients suspected of HNPCC for expression at RNA level, the effects on splicing were evaluated by RT-PCR analysis and systematic sequencing: *MLH1*: c.306+5G>A and c.307-29C>A; *MSH2*: c.1661G>A, c.2634G>A; *MSH6*: c.100G>C, c.3425C>T and c.4004A>C.

We demonstrate that 3 of the 7 UVs analyzed affect splicing. The variant c.306+5G>A, a G to A transversion affecting position +5 of the splice donor site of *MLH1*/exon 3; the sequencing of this revealed a deletion of five nucleotides which gave rise to the aberrant splicing. The substitution *MSH2* (c.1661G>A) affecting the last nucleotide of exon 10, the change from guanine to cytosine makes the natural splicing site 50 (value 0.60) disappear, using a new donor cryptic site in position c.1580 (value 0.56), thus producing the deletion of 81 nucleotides in the mRNA. This point was confirmed by means of RT-PCR analysis, so that the normal fragment would have a size of 357bp and the aberrant fragment 246bp. We confirmed complete skipping of exon 15 for the mutation *MSH2*: c.2634G>A.

The pathogenic splicing mutations identified in this study will contribute to the assessment of „unclassified variants“ in genetic counselling.

P06.099 Molecular characterization of CNS embryonal tumors in early childhood: differences between medulloblastoma and atypical teratoid rhabdoid tumor (AT/RT).

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Atypical Teratoid Rhabdoid tumors (AT/RT) are highly malignant CNS tumors with an ominous prognosis that justifies an aggressive treatment. Due to their heterogeneous histology, IHC profile and some morphologic overlap with Medulloblastoma (MB), make an accurate diagnosis results to be a difficult challenge.

In order to characterize the molecular profile, we constructed a specific Tissue Micro Array (TMA) with a series of 30 formalin-fixed paraffin-embedded childhood brain tumours (22 MB and 8 AT/RT), from year

2003 to 2009, were acquired from the archives of the Pathology Department of H. Virgen del Rocío, Seville, Spain. Representative areas of each tumour were chosen.

We studied by Immunohistochemistry (IHC) Ki-67, sinaptofisin, enolase, GFAP, EGFR, INI-1, β -catenin and cyclin D1 and by FISH we studied EGFR, HER2, MYC, MYCN, 11p, 17p/17q, 6q status.

AT/RT group showed: 1) Loss of expression for INI-1 protein in all the cases; 2) Positive immunohistochemical nuclear expression of β -catenin and cyclin D1 in 62% and 90% of the samples respectively, while the group of MB was positive only in a 13% and 9% respectively; 3) 75% of AT/RT samples carried 6q deletion while only 18% of MB showed this alteration. In contrast 36% of MB showed gain of 6q. Amplification of HER2 and MYC/MYCN was observed only in MB group in the 22% and 36% of these samples.

These results suggest that molecular characterization could be a helpful tool for a more accurate diagnosis of these two tumors.

P06.100 Exome re-sequencing of 7 melanoma cell lines to identify “driver” mutations

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Melanoma is the most dangerous form of skin cancer. Ultraviolet light exposure is a well known environmental risk factor for this tumor. Although a few prevalent mutations have been identified, the full mutational spectrum that includes the “driver” mutations of melanoma is unknown. To better understand the molecular pathophysiology of melanoma we have used high throughput sequencing of array-selected exons to determine the mutational spectrum in the protein-coding fraction of seven melanoma cell lines, 5 unrelated and 2 derived from the same patient (before and after treatment).

We performed the exome selection using CCDS capture arrays (34 Mb, 20000 genes) on genomic DNA extracted from cell lines derived from metastatic tumors and from normal tissues of the same patient (EBV-transformed or non-transformed PBLs). An improved capture protocol results in 60% of DNA library fragments originating from coding exons. Exomes were re-sequenced using 2-3 lanes of 2x76 bp paired-end sequencing, providing 40-80 fold coverage per sample. More than 98% of SNPs recovered on SNP arrays were confirmed by re-sequencing. Somatic mutations were determined by comparison between the melanoma and normal cell lines for each individual. We observe that the most abundant class of mutations is C>T, a hallmark of exposure to ultra-violet light. In order to detect “driver” mutations, we compared somatic mutations between individuals and revealed a subset of genes affected by probably deleterious mutations. We are also investigating rare germline variants that are shared by the melanoma patients which may include alleles that predispose individuals to this disease.

P06.101 Copy number profiling of metastatic melanoma cell lines

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Melanoma is a malignant tumour arising from melanocytes (pigment producing cells) which can generate metastases throughout the body. Although one of the less frequent skin cancers, melanoma is responsible for most of the associated deaths. We are analyzing seven metastatic melanoma cell lines, as well as matched control cells, with 1) karyotype, high-resolution CGH and SNP analyses to study large-scale genomic variation; 2) ultra-high-throughput sequencing of exome-capture genomic DNA to discover mutations in protein-coding genes; 3) transcriptome profiling using 454/Roche pyrophosphate sequencing to

identify melanoma-specific expression patterns and splicing events; and 4) methylation analysis to uncover epigenetic changes in melanoma cells that contribute to melanoma-specific expression patterns. Here we present results from the karyotype, CGH and SNP analyses, as well as preliminary results from transcriptome sequencing. We examine the challenges and limitations of working with highly aneuploid cancer genomes, and propose appropriate analytical procedures. Our comprehensive copy number profiling is useful to stratify melanomas into sub-categories based on genomic amplification status (see Table). We demonstrate a link between genomic amplification and increased gene expression, and we have grouped genes according to their copy number profiles. We anticipate that characterisation of melanoma-specific aberrations at the genomic, transcriptomic and epigenetic levels will improve clinical diagnosis by identifying new candidate genes playing a role in melanoma malignancy.

| Melanoma subset | Number of genes and genome fraction affected by copy number aberrations (* regions $\geq 100\text{Kb}$) | | |
|------------------------|--|---------------------|---|
| | Number of melanomas | Deletions* | Amplifications* (copy number ≥ 5) |
| Large deletion | 2 | 612Mb 3778 genes | 12Mb 77 genes |
| Moderate amplification | 3 | 26Mb 200 genes | 217Mb 1548 genes |
| Major amplification | 2 | 25Mb 148 genes | 1978Mb 14312 genes |

P06.102 Multiple Endocrine Neoplasia (MEN), type IIA - case report

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MEN2 is a rare familial cancer syndrome caused by mutations in the Rearranged During Transfection (RET) protooncogene and is inherited as an autosomal dominant disorder. MEN2 is classified into three subtypes: MEN2A, MEN2B, and familial medullary thyroid carcinoma. The overall frequency is 1:30,000-50,000. Almost all MEN2A patients (95%) develop medullary thyroid carcinoma. It is generally the first manifestation and appears between the ages of 5 to 25 years (mean 15 years). We report on a Caucasian male with complains of general weakness and weigh loss (10 kg for 2 month). The patient's mother has been diagnosed at 47 as bilateral pheochromocytomas and struma nodosa and died at age of 51. In the index patient abdominal ultrasound examination with biopsy of adrenal glands and histological analysis revealed pheochromocytoma on right side; elevated plasma calcitonin concentration and US scan with biopsy of thyroid tissue detected medular thyroid cancer. The index patient has two elder brothers - (it turned out that one of them) had been surgically treated for medular thyroid cancer in the past; bilateral pheochromocytomas were detected in the family screening process. Presymptomatic identification of at-risk siblings led to the diagnosis of medular thyroid cancer and right sided pheochromocytoma in the eldest brother. MEN2A diagnosis was suspected and we performed sequencing of RET protooncogene. The genetic analysis of the family affected members revealed c.1902C>G, p.Cys634Trp heterozygous mutation. Unfortunately, two kids in the families of 2 of the brothers have also inherited the mutation. All adult family members were offered genetic counseling.

P06.103 Aberrantly expressed microRNAs indicate the pathways potentially associated with non-small cell lung cancer development

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MicroRNAs (miRNAs) are regulatory molecules that have been implicated in development of a number of different cancers. The mechanisms of altered expression and functional relevance of many dysregulated miRNAs are still unknown.

To investigate the role of miRNAs in lung cancer development, we examined the miRNA expression profiles in 40 I and II stage non-small

cell lung cancers (NSCLC) and 40 paired normal lung samples. We used Illumina MicroRNA Expression Profiling Panels which are able to detect 858 human miRNAs. Altered expression of several miRNAs was confirmed by individual TaqMan miRNA assays. We identified miRNAs miR-9, miR-149 and miR-205 as most significantly up-regulated and miR-648, miR-1264 and miR-512-5p as most significantly down-regulated miRNAs in NSCLC samples ($P < 10.6$). The molecular pathways potentially affected by dysregulation of these miRNAs were identified using online tool Diana mirPath. Significant enrichment of predicted targets was observed in pathways involved in adhesion, signaling and tumorigenesis, indicating that altered miRNA expression may have a causal role in lung cancer development.

P06.104 The inhibition of cell proliferation by a microRNA molecule, miR-145 in human prostate cancer.

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Prostate cancer is one of the most significant cancers of the men and there is a need to identify novel therapeutic approaches in this disease. MicroRNAs are new class of small RNAs as regulators of gene expression. Alteration in microRNA expression may play a critical role in tumorigenesis, androgen independency and recurrence after radical prostatectomy. Global microRNA expression studies revealed the key regulatory microRNAs in prostate cancer. We have shown a widespread deregulation of microRNA expression in human prostate cancer. miR-125b, miR-145 and let-7c are down regulated in prostate tumors as compared to normal prostatic tissues. miR145 is downregulated in prostate tumor samples and prostate cancer cell lines including PC3, DU145, LNCaP and LAPC4 but has relatively higher expression in normal prostatic epithelial cells. The ectopic expression of miR145 significantly inhibited the proliferation of prostate cancer cells in vitro. The molecular mechanism behind this change are being investigated. Possible targets could be SEMA3A and SOX9 as identified by gene expression profiling. More studies to elucidate the mechanism of this inhibition are in progress. In summary, our data showed that miR-145 has an anti-proliferative ability as tested in vitro in human prostate cancer cell lines, suggesting microRNAs as important molecules in prostate cancer tumorigenesis.

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P06.105 The MLH1, MSH2 and MSH6 mutations among familial colon cancer Russian patients

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For the first time MLH1, MSH2 and MSH6 gene mutations were analyzed among Russian patients. There were 67% of hereditary non-polyposis colon cancer syndrome cases with MLH1 and MSH2 mutations. The mutations mainly were in MLH1 gene. Only one mutation - deletion of 3T in MLH1 exon 16 (p.Lys618del3) was repeated (20% of MLH1 spectrum). It is interesting that all patients with this mutation had multiple primary neoplasms. This is often than under the other mutations near the significance value ($P=0.06$). The rest mutations were unique. Among 80 colon cancer families not corresponding to Amsterdam's criterion only 3 cases (~4%) with MLH1/MSH2 mutations were found. The MSH6 gene was analyzed among 17 colon cancer families that included endometrial cancer cases. There were two MSH6 mutations in the sample (12%). One of those - 1142insCCCA was not found in the data bases and available literature and, so, is new.

P06.106 Development of a workflow to detect variants on MLH1 and MSH2 genes, using the newest capillary electrophoresis platform the 3500 Genetic Analyzer

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The MLH1 and MSH2 genes belong to the mismatch repair genes family. Thus far more than 200 different variants have been characterized in each of the MLH1 and MSH2 mismatch repair genes. These mutations are not present in any particular hotspot or zone of the gene and include either nucleotide substitutions (missense, nonsense or splicing errors) or insertions/deletions (gross or small). Specifically the overwhelming majority of hereditary nonpolyposis colorectal cancers (HNPCC) are attributed to mutations in the genes MLH1 and MSH2 respectively, which allows them to be classified as tumour suppressor genes. Ongoing research on MLH1 and MSH2 genes mutations has underlined the need for simple mutation detection research protocol. The high number of variations, the gene length and the absence of a hot spot region make the automated capillary electrophoresis (CE) DNA sequencing the gold standard method for this research. It's a highly referenced and robust technique that delivers long read lengths and the ability to sequence anywhere from a few to several hundred samples in a single day. Here we present a MLH1 and MSH2 research protocol. This protocol was developed via a collaborative research study on 15 colorectal cancer samples using the Applied Biosystems 3130 Genetic Analyzer and the newest capillary electrophoresis platform, the Applied Biosystems 3500 Genetic Analyzer.

P06.107 Expression of multidrug resistance-associated protein 1 and lung resistance-related protein in breast cancer patients: Correlation with response to chemotherapy treatment

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Drug resistance is still a great obstacle to the success treatment of breast cancer. Molecular investigations on drug resistance have led to the discovery of genes coding for P-gp, multidrug resistance-associated protein 1, lung resistance-related protein and many others. Despite the intensive studies regarding the role of these genes in inducing drug resistance in cancer patients the data is still controversial and debated.

Therefore, in this study we attempted to investigate the possible correlation between multidrug resistance-associated protein 1, lung resistance-related protein and clinical response in women with breast cancer. We determined mRNA levels of MRP1 and LRP in tumor and adjacent normal tissues of 54 breast cancer patients by Real Time RT-PCR.

A statistically significant increase in MRP1 and LRP expression level was observed when tumor tissues were compared with normal breast tissues. Furthermore, MRP1 and LRP expression levels were significantly different in patients responding to chemotherapy compared to nonresponding patients. No relation between the expression level of either of these genes and clinicopathology markers was found.

Our results suggest that MRP1 and LRP in human breast cancer cells may affect the clinical response to treatment and determination of MRP1 and LRP (either alone or in combination) may be valuable for the prediction of the chemotherapy outcome in breast cancer patients which remains to be cleared.

P06.108 Expression of MDR1 and MRP1 in colorectal cancer patients: Correlation with response to chemotherapy treatment

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Drug resistance is still a great obstacle to the success treatment of breast cancer. Molecular investigations on drug resistance have led to the discovery of genes coding for P-gp, and multidrug resistance-associated protein 1 (MRP1). Despite the intensive studies regarding the role of these genes in inducing drug resistance in cancer patients the data is still controversial and debated.

Therefore, in this study we attempted to investigate the possible correlation between MRP1 and MDR1 expression level and clinical response in patients with colorectal cancer. Tumor and adjacent normal

tissues from cancer patients were assessed for the expression level of MRP1 and MDR1 by Real Time RT-PCR.

Here we present some of our data in regards to the role of MDR1 and MRP1 in clinical response to chemotherapy.

P06.109 Evidence for a founder effect for the common Caucasian p.Tyr179Cys and p.Gly396Asp MUTYH mutations in the Italian population

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Background: MUTYH-associated polyposis (MAP) is an autosomal recessive inherited disorder characterized by a high risk of polyposis and colorectal carcinoma (CRC). Although MAP-causing mutations are distributed across the *MUTYH* locus, a few population-specific mutations have been reported. The majority of changes found in the *MUTYH* gene are missense variants; among these, p.Y179C and p.G396D (previously annotated as p.Y165C and p.G382D) represent approximately 75% of *MUTYH* mutations found in Caucasians. The high frequency of these two mutations in Caucasian populations could result from either recurrent *de novo* mutational events or from founder effects.

Aim: To verify the presence of possible founder effects for *MUTYH* mutations p.Y179C and p.G396D in the Italian population.

Materials and Methods: Haplotype analysis, using a set of 16 markers (microsatellites and SNPs) spanning a region of ~6cM covering the *MUTYH* locus, was performed in 42 unrelated p.Y179C and p.G396D carrier families and in a set healthy control chromosomes, including nuclear pedigrees. Haplotypes were manually constructed to minimize the minimum number of recombinations. The distributions of allelic frequencies in normal and mutated chromosomes were compared by Chi-squared and two tailed Fisher's exact tests.

Results: We observed statistically significant differences in allele frequencies between normal and both p.Y179C and p.G396D chromosomes. In addition, p.Y179C and p.G396D chromosomes were characterized by an association with distinct haplotypes.

Conclusions: Our data support the hypothesis that the common p.Y179C and p.G396D mutations may have been transmitted by a common founder in the Italian population.

P06.110 Phenotypic variation in MUTYH-associated Greek polyposis patients

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MUTYH-Associated Polyposis (MAP) syndrome is inherited as a recessive trait and is characterized by low polyp burden and increased lifetime colorectal cancer risk. In Caucasians, two *MUTYH* mutations: p.Y179C and p.G396D constitute the majority of all *MUTYH* mutations identified. Ten families meeting the MAP phenotypic criteria were studied. Patients were presented with 10-100 colorectal adenomas, and in most cases family history does not show vertical transmission of polyposis. The mean age of developing colorectal cancer was 47 years. Analysis of the *MUTYH* gene revealed eight different germline mutations in seven polyposis families. Furthermore, the pathogenicity of a 13-base pair deletion, located on intron 6 of *MUTYH* gene, which was identified in two unrelated Greek MAP patients, was elucidated. The polyp number, as well as the onset of colorectal cancer varied amongst families studied. This is the first report of germline *MUTYH* mutations in the Greek population, which can explain a fraction of polyposis patients, which are APC negative. The pathogenicity of a rare pathogenic *MUTYH* mutation, possibly associated with a relatively severe MAP phenotype, has been elucidated. It is quite evident that each MAP case should be handled according to its distinct genotype, following a specific prophylaxis protocol to ensure cancer prevention. Furthermore, preliminary data reveal genetic heterogeneity of the Greek population, as well as lower prevalence of the two most frequent mu-

tations, p.Y179C and p.G396D, when compared to other Caucasian populations.

P06.111 Chromosome 11q aberrations and the tumor suppressor CBL in myeloproliferative neoplasms**

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Aberrations of the long arm of chromosome 11 (11q) have been frequently described in hematological malignancies. Recently it was shown that these aberrations often associate with mutations of the tumor suppressor CBL at 11q23. Using high-resolution SNP array genotyping of patients with myeloproliferative neoplasms (MPN) we identified a variety of 11q aberrations that predominantly but not exclusively targeted CBL. Two patients harboring 11q uniparental disomy (UPD) spanning the CBL locus carried a mutation in the CBL gene. For the first time we show, that a chromosomal gain of 11q amplifies a mutant CBL allele. As two patients with 11q deletions at the CBL locus did not carry mutated CBL on the remaining allele, loss of a single CBL allele (haploinsufficiency) may also be oncogenic in MPN. Patients with MPN often develop secondary acute myeloid leukemia (AML) and since CBL mutations have been previously described in AML we examined the association of CBL mutations with post-MPN AML. Sequence analysis of CBL in 309 MPN patients in chronic phase and post-MPN leukemia revealed an association of CBL mutations with leukemic transformation (P=0.0048). Not all aberrations that we detected on chromosome 11q were overlapping with the CBL locus. Thus, other 11q tumor suppressor genes may play a role in the evolution of a malignant cell clone in MPN. The role of chromosome 11 in MPN pathogenesis is likely more complex than previously recognized.

P06.112 A missense mutation of the NBS1 gene altering DNA damage response in a patient with familial early-onset breast cancer

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While screening candidate Breast Cancer (BC) genes in BRCA-negative early-onset BC patients, we detected a constitutional heterozygous missense mutation (V210F) of the NBS1 gene in a patient with BC diagnosed at age 29 and a family history of early-onset BC.

NBS1 encodes a protein, nibrin, involved in the repair of DNA Double-Strand Breaks. Biallelic NBS1 mutations cause Nijmegen Breakage Syndrome, characterized by microcephaly, growth retardation, immunodeficiency, chromosome instability, radiation sensitivity, and a predisposition to lymphoid malignancies. However, there is growing evidence of an increased cancer risk in heterozygous carriers of NBS1 mutations.

The V210F variant has been previously reported in a child affected by acute lymphoblastic leukaemia and in a patient with melanoma, but not, to our knowledge, in healthy controls.

To explore the effect of this variant on Double-Strand Breaks Repair, we performed an immunofluorescence assay on EBV-transformed B lymphocytes from the patient. The response to DNA damage following exposure to γ -radiations was evaluated in terms of number, intensity, and persistence of histone γ -H2AX positive nuclear foci.

A marked increase of the γ -H2AX signal, indicating an increase in the number and intensity of nuclear foci, was found in the cells of the patient, in comparison with a healthy, wild-type control and with a heterozygous carrier of the 657del5 mutation, the most common NBS1 mutation.

These preliminary results suggest that the V210F mutation may actually alter NBS1 function and raise the hypothesis of a role in the genesis of cancer. Further studies are ongoing to test this hypothesis.

P06.113 Neuroblastoma genetics: A great challenge for the Paediatric Oncologist

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Few tumors have engendered as much fascination for clinical and laboratory investigators as neuroblastoma. Neuroblastoma, the most common solid tumor in childhood, is derived from primitive cells of the sympathetic nervous system. Genetic abnormalities in tumor cells correlate closely with the clinical outcome. These genetic abnormalities include gains as well as loss of genetic material (MYCN amplification chromosome 17q gain and deletions of 1p). MYCN amplification is the first genetic marker to be used as a guide for therapeutic decision and remains the most relevant prognosis factor.

The primary aim of our study is to combine the use of molecular genetics strategies to screen chromosomal rearrangements in Tunisian children neuroblastoma. For this we have chosen 3 techniques: chromogenic in situ hybridization (CISH), multiplex ligation probe amplification (MLPA) and microsatellite analysis, on fresh and paraffin-embedded tissues. We analyzed 22 neuroblastoma tumors encompassing 2 case of recidivism. Using both CISH and MLPA, we analyzed MYCN amplification rate. MLPA also allowed us to analyze MYCN nearby genes (NAG, DDX1 and ALK) and 17q gain, whereas microsatellite markers analysis enables us to identify 1p deletions. We investigated 21 patients, the age of tumor discovery ranged from 4 days of life to 10 years. MYCN amplification rate ranged from 5 to 9 copies, at least one patient showed chromosome 1p deletion. Amplification of MYCN nearby genes was frequently found associated to MYCN amplification. Our results correlates with patient prognosis and allowed us to set up the first Tunisian neuroblastoma therapeutic strategy based on molecular findings.

P06.114 Characterization of amplicon junction sequences in genomic regions surrounding the MYCN gene in neuroblastoma tumors; Implications for clinical follow-up of high-risk patients

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Background: Amplification of the MYCN (2p24) gene region is a common feature among one group of unfavorable neuroblastoma tumors. The aims of this study were to characterize the amplified DNA segments (amplicons) in detail and to obtain tumor-specific PCR fragments to be used for minimal residual disease (MRD) detection in these patients.

Method: High-density SNP-arrays were used to map the endpoints of the MYCN amplicons in a subset of neuroblastoma tumors. As we sought to clone novel junctions between amplicons, outward-facing primers were designed, giving rise to a PCR product only in the case of a rearrangement. DNA sequencing revealed information about the junction region and the successful primersets were also tested for MRD detection in a semi-quantitative PCR assay, comparing DNA from blood samples with a serial dilution of tumor/control DNA to estimate the amount of tumor DNA in the blood.

Results: A tumor-specific junction fragment was detected in each of the four cases hitherto analyzed, albeit different in each case. The junctions consisted of small microhomology regions of only a few bases and mapped to the reference genome as two separate hits on either side of MYCN, confirming a head-to-tail orientation of the amplicons. Our approach to MRD detection was found to be sensitive enough to detect the junction fragment in a 1/10⁶ dilution of Tumor/Control DNA and we were also able to estimate the tumor-DNA content in the patient samples. Thus, this method is suitable for patient-specific monitoring of treatment response and early detection of relapse.

P06.115 The importance of Trk gene family in neuroblastoma

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Neuroblastoma is the most frequent extracranial solid cancer in childhood and the most common cancer in infancy. Despite multimodal treatment: chemotherapy, surgery, radiotherapy, the prognosis of neuroblastoma is reserved. The Trk family genes play an important role in neuroblastoma behaviors. Neuroblastomas expressing TrkA are biologically favorable and evolve to spontaneous regression or differentiation; meanwhile TrkB-expressing tumors often have a poor outcome. Between 2000 and 2009 a total of 17 patients with Shimada grade III and IV neuroblastoma were admitted in our hospital. There were 7 boys and 10 girls, age between 3 months- 8 years. Most of the patients (11) were in stage IV, 4 were in stage III and 2 were IVS. Overall mortality rate was 77%, 75% in the Shimada III group, 81% in the Shimada IV group and 50% in the IVS. These rates are significantly higher than that previously mentioned in the literature and, more important, are similar for the Shimada III and IV. This is an indication that more is to be done for a better stratification of the patients. A better stratification means better results with fewer side effects. This is particularly important because the majority of survivors of high-risk neuroblastoma have long-term side effects from the treatment. Survivors of intermediate and high-risk treatment often experience hearing loss, growth reduction, thyroid function disorders, learning difficulties, and greater risk of secondary cancers. The determination of Trk A and Trk B improve risk grade stratification, therapy approach providing attractive targets and decrease side effect of chemotherapy.

P06.116 Multiplexed amplicon sequencing of the breast cancer genes BRCA1&2: challenges, opportunities and limitations.**

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Since the launch of 454 Next Generation Sequencing (NGS), an increasing number of applications has been published, however, its implementation in a diagnostic setting remains a challenge.

We first optimized an efficient workflow allowing pooling of as many patients possible in a single run. After amplification with amplicon-specific primers, a second reaction is performed to fuse the A/B adaptors and MID tags to each amplicon. Different set-ups for BRCA1/2 amplicon sequencing were used to evaluate coverage, sensitivity and specificity. Equimolar pooling of 112 simplex PCR reactions, covering the complete coding and splice site regions of BRCA1/2, was compared with equimolar pooling of these 112 amplicons in 16 strongly optimized multiplex reactions. Data analysis was performed with the VIP (Variant Identification Pipeline) software package.

More uniform coverage was obtained with the multiplexing approach. After optimization, a fold difference to mean coverage of 2 was reached for 90% of the amplicons. Sensitivity was evaluated with 132 distinct sequence variants of which 90 were deletion/insertion mutations. In total 129/132 (~98%) of all variants could be easily detected. Variants not detected were all deletions/insertions in homopolymeric repeats. By defining filters initial specificity for 4013 amplicons was increased from 50% to 98.5%.

By using a multiplex barcoding approach NGS becomes competitive with other prescreening techniques currently used in diagnostics. To our knowledge this is the largest validation of 454 amplicon pyrosequencing published so far and our approach can be used as a guidebook for the implementation of other diagnostic tests.

P06.117 PCR-based targeted sequence enrichment for next generation sequencing platform

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Many human diseases are associated with genetic polymorphisms. Resequencing candidate regions can provide valuable information about the genetic basis for these diseases. Combining Next Generation Sequencing with a new PCR-based enrichment method gener-

ates a robust and cost-effective workflow for deeper interrogation of targeted genomic regions of interest for specific applications. Here, we report the use of Next Generation Sequencing with PCR-based enrichment to extract target regions from Yoruba DNA. We present an optimized and flexible workflow for library construction post PCR enrichment to emulsion PCR and sequencing on a Next Generation Sequencing platform. We demonstrate that this pipeline provides a useful solution for targeted resequencing applications.

P06.118 Next Generation Sequencing (NGS) in molecular diagnostics: evaluation of data analysis software tools and application to BRCA1/2 testing

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One of the challenges in genetic testing is the implementation of the Next Generation Sequencing (NGS) technologies. There are technical and managerial pitfalls as well as aspects of validation and accreditation of both the 'wet' work and the data analysis. We develop diagnostic applications on the Roche 454-GS FLX platform, starting with BRCA1/2.

First, fragments spanning the coding region of the BRCA1 gene were amplified using different DNA samples, to generate an (artificial) control sample that contained 40 variations, including 16 frameshifts. The data files were analyzed with three commercial software tools: CLC Genomics Workbench (CLC bio), Sequence Pilot (SeqNext module, JSI medical systems) and GS Amplicon Variant Analyzer (AVA, Roche). Second, the coding regions of the BRCA1/2 genes were amplified for 454-GS FLX sequencing, using a recently developed multiplex assay (distributed by Multiplicon, Belgium).

All known tested variations could be detected by a combination of the different software tools; except for SeqNext, the tools missed one or a few variants. The variant frequencies (% mutant versus wild type reads) for heterozygous variations were 46 ± 2 . We are increasing the number of samples to generate figures for the sensitivity and reproducibility of the method.

The multiplex assay is robust for the simultaneous amplification of genomic fragments for subsequent analysis on the Roche platform. However, the software tools still have to undergo further developments. On the basis of the results using patient samples, we are confident that the multiplex PCR approach will soon be ready for introduction in molecular diagnostics.

P06.119 Analysis of NF2 mRNA expression in sporadic colon cancer

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NF2 protein product merlin is predominantly localized to the membrane-cytoskeleton interface and has a role in receiving extracellular signals and controlling cell-cell and cell-ECM communication, motility and differentiation. It has been shown recently that merlin inhibits Ras and Rac activation by uncoupling them from growth factor signals.

Since these processes are essential for tumorigenesis and metastasis in general, we decided to examine the possible role of NF2 in sporadic colon cancer through analysis of its mRNA expression. The NF2 gene (22q12) is composed of 17 exons, with two alternatively spliced major isoforms, type I and II. Isoform type I lacks exon 16, whereas isoform type II contains exon 16 but lacks exon 17. These isoforms are variably expressed in some tissue types with possibly different functions.

Real-time PCR was used to determine the NF2 mRNA expression, as well as expression of its mRNA isoforms type I and II in 60 pairs of colon cancer tumors and corresponding normal tissue. No statistically significant difference in total expression of NF2 gene as well as its isoforms I and II was observed in tumor tissue as compared to corresponding normal tissue. Results of mRNA expression were correlated with the patients' clinicopathological features. Expression of NF2 mRNA, as well as its isoforms I and II, was higher in moderately and poorly differentiated tumors and tumors classified as Dukes' C. Based on our results we can conclude that NF2 gene is involved in sporadic colon cancer development and progression.

P06.120 Analysis of genetic susceptibility to non-Hodgkin lymphoma (NHL) in Bashkortostan Republic of Russia

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NHL is a heterogeneous malignancy of B and T-cells that involves their uncontrolled clonal expansion in the periphery. The aim of study is to define the role of genetic polymorphisms and reveal potential susceptibility loci for NHL. The sample included 119 NHL patients aged 55-64 years from Bashkortostan. Patients were defined as all patients with a first, incident, morphologically verified diagnosis of NHL. Controls were matched for age, gender, ethnic origin and area of residence. We examined polymorphisms in homocysteine metabolism related enzymes (MTHFR C677T, MS 2756 AG), oxidative stress (MPO G-463A, SOD2 Val16Ala), detoxification (GSTM1*0, CYP1A1 A4889G, EPHX Tyr113His, CYP2E1 (intron6)), TNF- α (G-308A). No statistically significant effects on risk, incidence, NHL status with SOD2, MPO, CYP2E1, MTHFR, CYP1A1, GSTM1 genotypes were observed. Distribution of genotype and allele frequencies in TNF- α gene showed significant increase of TNF-308AA genotype and TNF-308A allele in cases. TNF-308AA genotype was more frequent in aggressive form (7,1%) than in indolent (0%). Individuals with TNF-308A allele, associated with higher constitutional and inducible expression of TNF- α , had a significant increase in NHL risk (OR=2,11, CI=1,14-3,92). Analysis of MS gene showed MS2756GG and MS2756GA genotypes to be associated with decreased risk (OR=0,38, CI=0,14-0,99). Significant differences in distribution of EPHX gene genotypes showed high possibility of aggressive NHL form development in individuals with His allele (OR=2,19, CI=1,23-3,93). Risk of NHL development was also higher in cohort aged less than 55 years old (OR=2,14, CI=1,01-4,56). These polymorphisms might play a role in NHL by influencing DNA synthesis or DNA methylation.

P06.121 Invasion and metastasis in non-small cell lung cancer: expression analyses

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Purpose: Non-small cell lung cancer (NSCLC) is major cause of cancer-related death. The poor outcome of NSCLC is usually caused by metastatic disease. Current diagnostics and treatment regimens are ineffective partly due to the limited ability to distinguish differences in inherent tumor invasiveness. The purpose of this study was to determine the expression levels of 84 genes associated with invasion and metastasis in Bulgarian patients with NSCLC.

Methods: Total RNA was extracted from 54 NSCLC samples and 34 adjacent non-tumorous lung tissues. Six pools were prepared - one control and 5 pools of tumor RNAs divided according to clinicopathological features including histological subtype and lymph node metastases (LNM). Expression profiles of 84 genes were determined by applying real-time PCR on pathway focused gene arrays (SABiosciences, USA).

Results: Common expression alterations in all 5 pools of NSCLC was upregulation of matrix metalloproteinases MMP11 and 13, their activator ETV4, as well as downregulation of TIMP3, cell growth and proliferation genes FGFR4 and TRPM1, cell adhesion gene MCAM and transcription factors NR4A3 and RORB. The genes underexpressed in LNM-positive samples are involved in cell adhesion (CDH6, ITGA7, SYK) and cell growth (SSTR2) while LNM-negative pool showed specific downregulation of matrix degradation related genes TIMP2 and CTSL1. Unlike adenocarcinomas squamous cell carcinomas showed prevalent deregulation of cell growth and proliferation genes, characteristic overexpression of MMP9, underexpression of adhesion gene MGAT5 and metastasis suppressor KISS1.

Conclusion: This study revealed distinct transcriptional profiles related to invasiveness and metastasis in pooled NSCLCs in respect to clinicopathological features.

P06.122 LNCR2 region and the incidence of polymorphic alleles in Czech patients with nonsmall cell lung cancer diagnosis

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Recent genome-wide association studies identified associations between the risk of lung cancer and certain single-nucleotide polymorphisms (SNP) mapping to the region LNCR2 on chromosome 15q including genes encoding the nicotinic receptors subunits. We have introduced the analysis of four SNPs in the vicinity of these genes: LOC123688 (rs931794, rs8034191), CHRNA3 (rs1051730) and CHRNA5 (rs16969968) to identify the frequency of these polymorphic alleles in Czech patients with nonsmall cell lung cancer (NSCLC) compared to two control group - individuals with non-neoplastic pulmonary diseases, and anonymized blood samples from routine laboratory. The DNAs of NSCLC patients were isolated either from peripheral blood leukocytes or from normal lung tissue.

We found no significant differences in the frequencies of SNP alleles between both subgroups of NSCLC patients regarding types of DNAs (PBL or tissue) investigated or between both control groups respectively. Generally, the individual alleles of SNPs in LNCR2 region form haplotypes, but a small proportion of "recombinants" could be detected; they are more frequent in the NSCLC patients than in control individuals. We looked for the association between haplotype variants in LNCR2 region and diagnosis of NSCLC. The highest difference in genotypic frequencies was seen between the groups of NSCLC patients and individuals with non-neoplastic pulmonary diseases, namely in rs8034191 and rs931794 ($P = 5.9 \times 10^{-6}$ and $P = 1.1 \times 10^{-6}$ respectively). Similar results have been obtained for smoking habitus.

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P06.123 Prognostic significance of NOTCH1 and FBXW7 mutations in pediatric T-ALL

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The NOTCH signalling pathway plays important role in the development of multicellular organisms, as it regulates cell proliferation, survival, and differentiation. NOTCH1 and/or FBXW7 mutations both lead to activation of the NOTCH1 pathway and are found in the majority of T-ALL patients.

In this study, mutation analysis of NOTCH1 and FBXW7 genes were performed in 87 pediatric T-ALLs who were treated on the ALL-BFM protocols. Overall, 22,2% of our T-ALL patients had NOTCH1 mutations and 10% FBXW7 mutations. We define novel mutations as well as previously reported ones inside the NOTCH1 and FBXW7. Whereas FBXW7 mutation frequency is similar to previous studies, prevalence of NOTCH1 mutation was relatively lower in our group. We also analyzed the relationship of the mutation data with the clinical and biological data of the patients. NOTCH1 and FBXW7, NOTCH1 alone were found correlated with lower initial leucocyte counts which was independent from sex and T- cell immunophenotype. However, NOTCH1 and FBXW7 mutations were not predictive of outcome in the overall cohort of pediatric T-ALLs.

The characterization of the NOTCH1 mutations and certain targets on the NOTCH pathway are important for developing novel and specific therapeutic strategies. Inhibitors of γ -secretase have recently been tested in T-ALL cell lines and were shown to induce cell cycle arrest. Importantly, FBXW7 mutations have been associated with resistance

to γ -secretase inhibitor treatment. Therefore, the identification of NOTCH1 and FBXW7 mutations need to be taken into account when choosing target therapies in T-ALL patients.

P06.124 Nucleophosmin is overexpressed in thyroid tumors

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Nucleophosmin (NPM) is a protein that contributes to several cell functions. Depending on the context, it can act as oncogene or tumor suppressor gene. Misexpression or delocalization of NPM, associated with mutations in the structural gene, has been described in many human neoplasia. Expression and localization of NPM was investigated in human thyroid tumors and cell lines. By immunohistochemistry studies, NPM overexpression was detected in papillary, follicular, undifferentiated thyroid cancer and also in follicular benign adenomas, indicating it as an early event during thyroid tumorigenesis. In normal thyroid FRTL-5 cells, a positive correlation between NPM protein levels and proliferation state was detected. By using quantitative RT-PCR to measure NPM mRNA, we found that overexpression of NPM protein is not always associated to increase of NPM transcript, suggesting the existence of a post-mRNA regulatory mechanism. Finally, while in non-cancerous thyroid cell lines NPM is localized in nucleolus, in some thyroid tumor cell lines also a nuclear localization was detected, suggesting that in thyroid tumors a partial delocalization of NPM may occur. However, no *NPM1* gene mutations were detected in all except one thyroid tumor examined.

P06.125 Clinical and cytogenetic characteristics of acute leukemias with t(9;11)(p22;p15)

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The *NUP98* gene in 11p15 is fused to a wide variety of translocation partners in hematologic malignancies, with many of the corresponding chromosomal changes (most often translocations) being reported in only a few cases each. One of these rare translocations is t(9;11)(p22;p15), which has been found in four cases of acute myeloid leukaemias (AML), in one case of acute biphenotypic leukaemia, and in one chronic myeloid leukaemia (CML) in myeloid blast crisis (Mitelman et al, 2009; present study). By reviewing the available clinical data in these cases, including one from our own department, some notable features are emerging, albeit based on a small number. The t(9;11) was the sole cytogenetic aberration in all cases, except for the CML, in which the typical CML-related t(9;22)(q34;q11.2) was present as well. All cases investigated molecularly harboured the *NUP98-LEDGF* fusion. Two cases were males and four were females, and all but one were adults (the exception being a 5-year-old girl with AML M2/M3). Excluding this pediatric patient, the median age at diagnosis was 52 years (20-64). The median peripheral blood values were hemoglobin 89 (72-104) g/l, platelets 42 (30-104) $\times 10^9/l$, and the white blood cell count 64 (1.4-293) $\times 10^9/l$. Among the adult acute leukemia patients, all relapsed within five years (3-54 months), suggesting an unfavourable prognosis.

P06.126 Study of opticin expression in human cancer cell lines

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Introduction: Opticin is a member of a family of secreted proteoglycans that mainly expressed in human eyes. It is also expressed in human brain, ligament, liver and skin, but at lower levels than the eyes. The

precise function of optisin are unknown but may be involved in fibrillogenesis of collagen molecules to form the vitreous gel and is potentially involved in maintaining the spacing between the collagen fibrils of the tissue. Here we present expression profile of optisin in a variety of human cancer cell lines.

Methods: Two peptides from extracellular domain and signal peptide of optisin were synthesized and used for polyclonal antibodies production. Anti-optisin polyclonal antibodies were purified and evaluated by ELISA, and Western blot (WB). Optisin expression in mRNA and protein levels were studied by RT-PCR and Western blot on the following cell lines: A-172 (brain cancer), T47D (mammary gland carcinoma), Paca-2 (pancreas carcinoma), Ej-138 (bladder carcinoma), Calu6 (lung carcinoma), ACHN (kidney cancer), SKOV3 (ovarian cancer), LS-180 (colon carcinoma), A-375 (skin cancer), PC3 (prostate adenocarcinoma), CLL-CII (chronic lymphocytic leukemia).

Results: All cancer cell lines except A-172 expressed optisin at both mRNA and protein levels.

Conclusion: Our finding may represent optisin as a novel tumor marker in a wide variety of cancers.

P06.127 Gene polymorphisms that affect the circulating amount and cytokine function of leptin are associated with risk for oral cancer

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Introduction: Functional DNA polymorphisms in genes of factors regulating cell proliferation have been associated with increased predisposition for oral cancer by genetic association studies performed by our group and others. This study examined the possible association of oral cancer risk with DNA polymorphisms -2548G/A and Q223R in the leptin (LEP) and leptin receptor (LEPR) genes, respectively. Both polymorphisms affect the circulating amount and cytokine-type function of leptin, which in the oral region seems to promote keratinocyte proliferation.

Methods: PCR-based RFLP analysis was performed in DNA samples of 150 patients with oral squamous cell carcinoma (OSCC) and 152 healthy controls of equivalent gender, age, and ethnicity (Greeks and Germans).

Results: In comparison to controls, the homozygous high gene expression genotype A/A of the LEP -2548G/A polymorphism was significantly increased in the subgroups of patients with advanced cancer stages ($P = 0.0001$; OR 9.0, 95% CI 2.62-30.89), of patients with a positive family history of cancer ($P = 0.0346$; OR 3.55, 95% CI 1.15-11.01) and nonsmokers ($P = 0.0051$; OR 9.69, 95% CI 1.03-91.24). The homozygous low-leptin-binding genotype G/G of the LEPR Q223R polymorphism was strongly associated with an increased risk for OSCC for all patients ($P = 0.0028$; OR 4.11, 95% CI 1.30-12.97) as well for most of the patient subgroups.

Conclusion: These findings reveal a significant contribution of circulating leptin in the occurrence of OSCC. This is the first study indicating the association of LEP and LEPR gene polymorphisms with increased risk for oral cancer.

P06.128 P38 gene mutations and Breast cancer

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It has been known for a while that the P38 protein induces cell death. On the other hand it has been demonstrated that P38 can be essential for death receptor-mediated apoptosis in cancer cells.

In this study a kind of phenotype-genotype research for breast carcinoma and P38 gene mutations has been done.

The correlation between breast cancer stage and its prognosis has been evaluated with the mutation types of the P38 and their statistical analyses were the matter of interest through a series of clinical and genetic variables.

Analyses were conducted for the 400 patients and 160 controls genotyped for P38, including 182 patients, 76 Control of premenopausal women and 218 patients, 84 Control of postmenopausal women and ages were 35-55 years. The P38 gene consists of 12 exons.

Searching for mutations, we performed SSCP-PCR protocol. Sequenced with a DNA sequencer.

Our study shows any P38 mutation database phenotypically interrelated with the clinical prognoses.

P06.129 Comparative yield of endosonography and magnetic resonance imaging in surveillance of individuals at high risk for pancreatic cancer

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Introduction: Individuals at high risk for pancreatic cancer (PC) might benefit of a surveillance-program diagnosing this disease at an early and potentially curable stage. Endosonography (EUS) has proved to be a potentially valuable tool for surveillance-purposes. Data for MRI are limited. We present preliminary results of a comparative study between baseline-EUS and baseline-MRI investigations in high-risk individuals (HRI) entering a yearly surveillance-program.

Methods: Individuals eligible for surveillance were 1st-degree members of families with familial-PC (FPC) and mutation-carriers of PC-prone hereditary syndromes (e.g. p16-Leiden, BRCA1/2, p53, Peutz-Jeghers syndrome (PJS)). HRI prospectively underwent EUS and MRI.

Results: Sixty-four HRI (M/F 30/34, median age 50 years) underwent baseline surveillance-investigations (42.2% FPC, 28.1% p16-Leiden, 6.3% BRCA1-mutation, 17.2% BRCA2-mutation, 3.1% p53-mutation, 3.1% PJS). Asymptomatic masses (50mm, 10mm and 7mm) were detected in three individuals (4.7%; 2 p16-Leiden, 1 FPC). All masses were detected by EUS only (in one individual MRI was contraindicated), of which two proved to be a malignancy and in one no malignancy but focal areas of premalignant-lesions were found. Small cystic lesions (median size 4.5mm) were detected in 15 individuals (23%), more often by MRI (MRI/ EUS 16/12). None showed signs of malignancy.

Conclusion: Based on these preliminary baseline-results, EUS and MRI seem complementary techniques to detect (potential) (pre)malignant lesions in HRI. All mass lesions were detected by EUS only, including two malignancies and one lesion with focal areas of premalignant-lesions. Small cystic lesions were frequently found (23%) and more often detected by MRI. Whether surveillance improves survival remains to be investigated.

P06.130 Genetic analysis of BRAF mutation status in papillary thyroid carcinoma

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Thyroid cancer represents a serious problem worldwide. Progress in human genome project and modern molecular biology techniques have improved our understanding of the genetic changes that lead to carcinogenesis and have provided opportunities for identifying disease biomarkers. The recently discovered activating mutation in the gene for the B-type Raf kinase (BRAF) is the most common genetic alteration in thyroid cancer. The BRAF mutation (V600E) is the most frequent genetic alteration in papillary thyroid carcinoma (PTC). The role of BRAF mutation as a poor prognostic factor has been reported in many studies. 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.

The aim of our study was to establish the methods for rapid, sensitive and cheap detection of BRAF mutation status in tumour tissues DNA. We applied DxS BRAF mutation test kit to detect somatic mutations in BRAF gene in the set of 70 samples. Next step was to evaluate the SNaPshot analysis and compare the sensitivity of these two methods. Our results demonstrate the utility of using the SNaPshot analysis for detection of somatic BRAF mutations in clinical samples. The sensitivity of mentioned test is comparable to commercial DxS BRAF mutation test kit.

P06.132 Role of p73 and acetylation of H3 histone in the regulation of periostin expression in thyroid cancer cell lines

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Periostin expression is a characteristic of the epithelial-mesenchymal transition, which occurs during epithelial tumor progression. Previous data indicate that periostin expression is related to aggressiveness in thyroid tumors. In order to identify mechanisms responsible for periostin expression during thyroid tumorigenesis, four different human thyroid tumor-derived cell lines were investigated by chromatin immunoprecipitation assay: BCPAP and TPC-1 (from papillary thyroid carcinoma), WRO (from follicular thyroid carcinoma), FRO (from undifferentiated thyroid carcinoma). Steady-state levels of acetylated histone H3 at lysines 9 and 14 are not related to periostin mRNA levels. Moreover, treatment of WRO and FRO cells with the histone deacetylase inhibitor, Tricostatin A, increases acetylated H3 levels at periostin promoter or coding sequence but reduces periostin mRNA levels. Therefore, no relationship was observed between H3 acetylation status at levels of either periostin promoter and periostin expression.

In addition to epigenetic mechanisms, by cell transfection, the effect of several transcription factors was investigated. The thyroid-specific transcription factors Pax8 and Hex have no effects on periostin promoter. Instead, Δ Np73, but not TAp73 α TAp73 β , significantly increases periostin promoter. Since Δ Np73 is expressed in thyroid cancer but not in normal thyroid tissue, our data suggest a molecular mechanism involved in development of thyroid carcinomas.

Moreover, our results suggest that periostin gene expression is controlled in cancer thyroid cells by mechanisms that are independent from the levels of H3 histone acetylation.

P06.133 Molecular genetic analysis of apparently sporadic pheochromocytomas and paragangliomas in Czech patients

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Pheochromocytoma is sympathetic tumour of chromaffin cells in the adrenal medulla that may produce and secrete catecholamines. This rare endocrine disorder causing arterial hypertension among approximately 0,1 % of patients with hypertension, occurs in approximately 90 % 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 Krebs 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 187 sporadic pheochromocytoma patients 5 germline mutations were found in the VHL gene. Further, 8 mutations in SDHB gene were detected. In addition, in 3 examined patients with paraganglioma we detected mutation of the start codon in SDHD gene.

P06.134 PRDM16 is frequently rearranged with various partner genes in myeloid malignancies with 1p36 alterations

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Cytogenetic rearrangements of chromosomal band 1p36 are found in approximately 0.1% of myeloid malignancies. PRDM16, a gene located on 1p36.32, is involved in the reciprocal translocations t(1;3)(p36;q21) and more rarely t(1;21)(p36;q22) in myelodysplastic syndromes (MDS) and acute myeloid leukemias (AML).

We studied 83 myeloid malignancies with 1p36 abnormalities by fluorescent *in situ* hybridization (FISH) with a bacterial artificial chromosomes (BAC) contig containing more than 80 BAC probes on 1p36. In this series, PRDM16 was found to be rearranged with RPN1 in 30 cases of t(1;3)(p36;q21), AML1/RUNX1 in 1 case of t(1;21)(p36;q22), TEL/ETV6 in 1 case of t(1;12)(p36;p13), IKZF1 in 1 case of t(1;7)(p36;p12), CDH4 in 1 case of add(1)(p36), NSF in 1 case of t(1;17)(p36;q21), a non-coding unknown sequence in 1 case of t(1;2)(p36;p12), and 2 further loci in 2 cases of t(1;2)(p36;p21).

PRDM16 was thus involved in over 45% of myeloid malignancies with 1p36 rearrangements. Like MDS/EVI-1, PRDM16 encodes for a zinc finger transcription factor and contains an N-terminal PR domain. 2 isoforms have been described. Most translocations would allow for the expression of both the long and the short isoform (lacking the PR domain). This is in contrast with previously published studies which suggested that, as for MDS/EVI-1, only the short isoform was supposed to have an oncogenic effect due to its translocation-induced upregulation in AML. Further studies are needed to understand the pathogenesis of AML and MDS mediated by PRDM16 isoforms and the role of the partner genes.

P06.135 Polymorphic variants in CYP1B1 linked with increased risk for prostate cancer in Bulgarian patients

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Introduction: Prostate cancer (PC) is the most diagnosed non-skin cancer and it is the second leading cause of cancer death in men. One of the genes associated with PC is CYP1B1 which encodes cytochrome P450 1B1. This enzyme activates many carcinogens and catalyzes hydroxylation of estrogens. It is over expressed in tumors and some polymorphic variants may increase the activity of the enzyme and have role in human prostate carcinogenesis.

Materials and methods: We have investigated the association with PC of four polymorphisms in exons 2 and 3 in CYP1B1 in case control study of 181 PC patients and 200 controls. Direct sequencing was used for genotyping and SeqScape for scoring.

Results: Positive association with PC risk showed the polymorphic variants rs1056836 (L432V) and rs1056837 (D449D). The C/C genotype of rs1056836 and rs1056837 have OR=1.44, 95% confidential interval (CI)=0.95-2.18, p=0.053. The C allele of the same variants

shows OR=1.345, 95% CI= 1.002-1.8, p=0.028. The polymorphisms rs1056827 (A119S) and rs1800440 (N453S) did not show any significant association with PC. No association was found between stage and grade of cancer with any of the polymorphisms.

Discussion: *CYP1B1* is hypothesised to play an important role in carcinogenesis owing to its role in the metabolism of both environmental and endogenous procarcinogens. Several studies have demonstrated the association between one or more genetic variants of *CYP1B1* and increased PC risk.

Conclusions: *CYP1B1* polymorphisms D449D and L432V showed association with PC in the Bulgarian population but further study with larger sample size is needed.

P06.136 Study of Ccassettes alterations in Mitochondrial D-Loop in Iranian prostate cancer patients

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Prostate cancer which is the most commonly diagnosed nonskin malignancy among men has heterogenous nature that multiple genes may involve in its occurrence and progression. Mitochondrial genomic mutations are found in variety of human cancers. The non-coding displacement-loop(D-loop) region of mtDNA in which contains essential sequences for the initiation of replication and transcription, is a polymorphic region that accumulates point mutations. Mt D-Loop has two hypervariable regions(HS VI, HS VII) with homopolymeric C. To investigate the mitochondrial micro satellite instability (mtMSI) within the mononucleotide C repeat at np 303-312 and 16184-16195 of mt D-Loop hypervariable regions in Iranian prostate cancer patients and Benign prostatic hyperplasia(BPH) group, DNA from 30 pathologically-confirmed prostate cancer patients (paraffin-embedded tissues of tumour and adjacent normal tissues) and 30 age-matched with BPH extracted and amplified D-Loop region by PCR. Sequencing results showed different alterations in two C cassettes. Most of them were as follows: del C, C→A and C→T at np 16184-1695, del and ins C in homopolymeric stretch interrupted by a T at np 303-312. The identification of micro satellite instability may complement other early detection approaches for prostate cancer and BPH.

P06.137 Paradoxical apoptotic induction of MCS-C2, a pyrrolo-pyrimidine derivative, via up-regulation of p53-independent, AR-dependent p21^{CIP1} expression in androgen-dependent prostate cancer cells

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Androgen is essential for prostate development and homeostasis. Androgen, acting through androgen receptor (AR), regulates not only a series of androgen target genes, such as PSA, but also genes for cell cycle- and apoptosis-regulatory molecules within prostate epithelial cells, such as p21^{CIP1}, which induces cell cycle arrest in response to DNA damage and protects cancer cells against p53-mediated apoptosis.

In the course of screening for novel modulators on apoptotic induction, we generated MCS-C2, a pyrrolo-pyrimidine derivative. MCS-C2 induced cell growth inhibition and apoptosis in a time- and dose-dependent manner in androgen-independent prostate cancer cells (DU145, PC3) and various non-prostate cancer cells. However, MCS-C2 paradoxically induced apoptosis at specific drug concentration (6 μM) in androgen-dependent prostate cancer cells (LNCaP, LNCaP-E9, -G4 and C4-2), while weakly inducing apoptosis at lower (3 μM) and higher (9, 12 μM) drug concentration.

To investigate the molecular mechanisms involved in this paradoxical apoptotic induction of 6 μM MCS-C2 in LNCaP and its subline cells, we performed real time-CEs, Western blots, real time-PCR, confocal microscopic analysis, knocking-down using siRNA.

Interestingly, paradoxical apoptotic induction of 6 μM MCS-C2 is associated with dramatic up-regulation (46-fold) of p53-independent, AR-dependent p21^{CIP1}.

Androgen, in general, up-regulates expression of p21^{CIP1} gene in stimulating prostate cancer cell proliferation. However, in contrast, we conclude that up-regulation of 6 μM MCS-C2-mediated, p53-independent p21^{CIP1}, which is activated by AR via a canonical androgen response

element (ARE) in its proximal promoter region, plays pivotal role in the cellular signalling pathways that control apoptosis of androgen-dependent prostate cancer cells.

P06.138 Multifaceted preventive effects of single agent quercetin on a human prostate adenocarcinoma cell line (PC-3): Implications to nutritional transcriptomics and multi-target therapy

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The aim of the present study is to evaluate the effects of quercetin, a dietary flavonoid, on human prostate adenocarcinoma PC-3 cells. Lactate dehydrogenase (LDH) release, microculture tetrazolium test (MTT assay) and real-time PCR array were employed to assess the influences of quercetin on cell cytotoxicity, cell proliferation and expression of various genes in PC-3 cell line. Quercetin inhibited cell growth and proliferation and modulated the expression of genes involved in DNA repair, matrix degradation and tumor invasion, angiogenesis, apoptosis, cell cycle, metabolism and glycolysis. More importantly, quercetin inhibited the expression of genes responsible for progression from the androgen deprivation-responsive stage to the hormone deprivation refractory phase. In addition, no cytotoxicity of quercetin on PC-3 cells was observed. Taken together, as shown by the issues of the current study for the first time, the manifold inhibitory impacts of quercetin on PC-3 cells may introduce quercetin as an efficacious „magic shotgun“ in order to be used in the future nutritional transcriptomic investigations and multi-target therapy to overcome the therapeutic impediments in crusade against prostate cancer.

P06.139 GSTP1 CpG island hypermethylation as an epigenetic biomarker in the molecular detection of prostate cancer

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Introduction: Prostate cancer represents a leading cause of cancer related mortality and mortality among men.

GSTP1 promoter hypermethylation occurs during carcinogenesis and is considered to be a major event of prostate carcinogenesis. DNA-based biomarkers are a class of new and promising tools for the early cancer detection.

The aim of our study was to detect the promoter hypermethylation of GSTP1 gene in blood and tissue samples from patients with PCa and BPH. To detect this epigenetic DNA alteration we applied the methylation specific PCR (MSP) method.

Materials and methods: For our study we used tissue and blood samples from 57 patients with the histological diagnosis of PCa, with a Gleason score of 4 to 7, and 44 patients with the diagnosis of BPH.

Patients with prostate cancer were subdivided according to their Gleason score, PSA, age and TNM staging.

Results: GSTP1 promoter hypermethylation was detected in 55 from 57 prostate cancer samples (96,5%) but it was not detected in any sample from patients with benign prostatic hyperplasia.

Conclusion: Promoter hypermethylation of GSTP1 gene distinguishes between PCa and BPH and therefore, this epigenetic alteration can be used as a biomarker for screening, early diagnosis and molecular staging of prostate cancer.

P06.140 Investigation of PTCH1 promoter mutations and polymorphisms

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PTCH1 is a tumor suppressor gene, located at 9q22.3, encoding a 12-pass transmembrane glycoprotein, that acts as an antagonist in the Hedgehog signaling pathway. PTCH1 gene has 23 coding exons and several alternative forms of exon 1.

PTCH1 is often mutated in Gorlin syndrome and various tumors, basal cell carcinomas in particular. Gorlin syndrome is a rare autosomal dominant disorder characterized with multiple basal cell carcinomas (BCCs), medulloblastomas, meningiomas, ovarian fibromas, jaw cysts, different developmental abnormalities, such as craniofacial alterations, bifid ribs, and polydactyly and syndactyly.

Basal cell carcinoma (BCC) of the skin, the most common human can-

cer, shows a continuously increasing incidence, occurring predominantly on sun-exposed skin of elderly fair-skinned people. Several tumor suppressor genes and oncogenes have been implicated in the pathogenesis of BCCs, and most of them are members of the Hedgehog signaling pathway.

In our previous research we discovered several new PTCH1 mutations and polymorphisms located in promoter region of exon 1b. Two new mutations: c.-892_-891CC>TT and c.-808C>T were discovered in BCCs and, new polymorphism c.-1184G>A was found in screening of Gorlin syndrome samples, healthy controls, BCCs and ovarian tumors. Additionally, two new alleles with 5 and 6 CGG repeats were discovered in the CGG repeat polymorphism in the 5'UTR region.

We are continuing this research with the functional impact of these mutations and polymorphisms on the promoter activity and consequently on PTCH1 role in the pathway.

P06.141 Next Generation Presequencing for *BRCA1* and *BRCA2* Genes : comparison between qPCR-HRM and EMMA

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Most are involved with next generation sequencing research projects. However, two new routine methods (qPCR-HRM and EMMA) provide valuable information to screen point mutations and large rearrangements at low cost and low computing investment. We compare them prospectively on 91 patients with a familial cancer predisposition.

METHOD : qPCR-HRM was performed with 79 amplicons, in triplicate, on a LightCycler480 (Roche Diagnostic). EMMA was performed with 24 amplicon multiplexes (Fluigent), on an automatic sequencer ABI3130XL (Applied Biosystem). Softwares were GeneScanning (Roche Diagnostic) and home-made macro in qPCR-HRM and Emmalys (Fluigent) in EMMA.

RESULTS : Total number of analyzed amplicons was 7189 for qPCR-HRM and 7371 for EMMA. Abnormal profiles were 730 in qPCR-HRM and 1141 in EMMA. There were 16 deleterious mutations (17,5%) and 15 unknown variants (UV), a duplication (exons 5-7) and a deletion (exon 24) in *BRCA1* and none in *BRCA2*. All events were detected by the two approaches.

DISCUSSION : Their advantages are discussed. For 8 samples, a run is 9 hours in qPCR-HRM and 12 hours in EMMA. qPCR-HRM sounds more rapid and appropriate for urgent screening. There were no maintenance, no technical skill in automatic sequencer, PCR products for direct sequencing and ability to rapidly shift to new primers or genes. EMMA can genotype current polymorphisms. Then, a validated multiplex kit clearly lessen the number of sequences. Emmalys software is well-dedicated to diagnostic purposes and large series.

CONCLUSION : Both methods are similar high throughput pre-screening for point and complex mutations in *BRCA1/BRCA2* genes.

P06.142 Molecular genetic alterations in the *VHL* gene and methylation of several tumor suppressor genes in sporadic clear cell renal cancer

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Renal cancer (RC) is one of ten most frequent carcinomas in adults and represents actual problem of modern oncology. Aim of this investigation is molecular genetic analysis of RC for development of potential markers of disease. We have studied 209 RC samples, 192 of which were clear cell carcinomas. Mutations in the *VHL* gene were tested using SSCP and sequencing, methylation was detected by methylsensitive endonuclease digesting and following PCR (hypermethylated samples were confirmed using bisulphite sequencing). We have detected *VHL* somatic mutations in 35.4% of samples. *VHL* inactivating events were present in 53.7% of patients with stage I that testified in favour for early alteration of this gene in clear cell RC. Aberrant methylation of *VHL* was observed in 12.0%, *RASSF1* - 56.0%, *FHIT* - 58.4%, and *CDH1* - 46.4% of cases. Methylation of at least one tested gene was detected in 84.1% of samples. *RASSF1* hypermethylation was associated with later RC stages ($p = 0.015$) and metastases ($p = 0.036$). Aberrant methylation of *CDH1* was associated with tumor progression, invasion, and metastases ($P = 0.009$, 0.039, and 0.002 correspondingly). Results of this investigation denoted the opportunity for using mutation and methylation of *VHL*, aberrant methylation of *RASSF1* and *CDH1* in RC molecular marker system.

P06.143 Phytoalexin resveratrol induces apoptosis in hormone-resistant cancer cell lines.

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Phytoalexin resveratrol has some structural similarities to diethyl stilbestrol, a synthetic estrogen. Resveratrol has been reported to possess cancer preventive properties.

In this study, we analyzed anti-tumor activity of resveratrol towards breast and prostate cancer cell lines.

Methods: We incubated two human cancer cell lines Du145, MBA-MD-231 (ER-negative) with resveratrol in high concentration, in dosage and time dependent manner; and analyzed the influence of drug on cell lines by flow cytometry.

Resveratrol treatment had reduced the proliferation of human cancer cells, cells had been arrested in the G2/M phase, and the percentage of cells in the subG1/G0 fraction had increased.

We characterized the anti-proliferation activities of resveratrol.

Treatment with resveratrol had resulted in a non-significant decreasing the percentage of cells in the G1/G0 phase in androgen responsive human breast cancer cell line (50-100 μ Mol/L for 24 hours), and dose-response (50-100 μ Mol/L) induction of apoptosis in androgen responsive human prostate cancer cells (incubation time 24 and 48 hours). However, at similar concentrations, resveratrol treatment did not affect the viability and rate of apoptosis in normal human prostate epithelial cells.

These data suggest that resveratrol have to be further examined as a potentially clinical chemotherapeutic agent for treating breast and prostate cancer.

P06.144 Modification effect of RET+3:T allele in medullary thyroid cancer

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Sporadic medullary thyroid cancer is a non-hereditary type of medullary cancer. Familial type is caused by mutation in the RET gene. The RET protooncogene is one of the receptor tyrosine kinases, cell-surface molecules that transduce signals for cell growth and differentiation. Medullary thyroid cancer arises from specialized cells named parafollicular cells. The present study concerns RET+3:T polymorphism localized in enhancer region. We investigated only sporadic cases of medullary thyroid cancer. Patients are delivered from Department of Endocrinology, Metabolism and Internal Diseases, University of Medical Sciences in Poznan. In our studies, we compared the frequency of the occurrence of the RET+3:T allele in our group of 48 non-familial MTC patients with the frequency of occurrence of the allele in the Polish population. The frequency of the occurrence of the heterozygote variant of the RET+3:T for the Polish population reached almost 12% (18/152) of heterozygotes but in the group of patients with MTC, we did not find even a single RET+3:T allele. The frequency difference is statistically significant and in the Fisher's Exact Test, the two-sided P value is 0.0080. This observation allows assuming that the occurrence of the RET+3:T in the heterozygotic state may lead to the inhibition of the disease phenotype in the cases of the medullary thyroid carcinoma.

P06.145 MDM2 and TP53 are modifier genes of retinoblastoma

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Current evidence support the role of DNA repair and apoptosis gene polymorphisms as cancer modifiers. Two common SNPs TP53 R72P and MDM2 SNP309 with known functional effects have been studied with contrasting findings in both sporadic cancer (gastric, lung, childhood ALL) and the inherited Lynch syndrome, and in Li-Fraumeni syndrome a significant interaction between the germline TP53 mutation and the MDM2 SNP has been shown. To investigate their role in hereditary retinoblastoma we genotyped the two SNPs by Pyrosequencing® assays on blood DNA of 90 patients with known germinal RB1 mutation, 34 familiar. A descriptive analysis showed an earlier age at diagnosis in patients with bilateral retinoblastoma than in those with unilateral retinoblastoma (median age: 0.57 yrs vs 1.49 yrs, respectively, p<0.001). Since age of onset is often not exactly known, we considered bilaterality as a more robust measure of the variable genetic risk. A multivariate logistic regression model adjusted for age and gender showed the risk of bilateral disease to be: i) as for the type of RB1 mutation higher for splicing and missense mutations than for deletions, duplications, nonsense and frameshift mutations but not significantly so (OR=1.33; 95% CI 0.22 - 8.22); ii) as for the MDM2 SNP309, significantly higher for the GG genotype than TT (OR=11.78, 95% CI 2.18 - 63.65) but not significantly for TG; iii) as for the TP53 R72P SNP not significantly for the PP genotype. Our results suggest for the first time that MDM2 and TP53 may be modifiers of Retinoblastoma as well.

P06.146 RB1 gene mutation in children with retinoblastoma

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Background: Retinoblastoma is the most common intraocular tumour in children. The molecular scanning of RB1 genes in searching of a germline mutation lead to the publication of new mutations whose knowledge is important for genetic counselling and the characterization of phenotypic relationships.

Results: RB1 gene mutation was first examined in child patients diagnosed with retinoblastoma as germline mutation detected in peripheral blood or bone marrow. Out of the entire number of patients, 14 cases out of 21 (66,6%) were diagnosed with unilateral retinoblastoma, and 7 out of 21 (33,3%) had bilateral retinoblastoma. In order to identify germline mutations we used the standard PCR, and for a proper visu-

alization we used *Denaturing Gradient Gel Electrophoresis* (DGGE). In case of positive findings we sequenced the particular fragments. In case of negative results we continued by using Multiplex Ligation - dependent Probe Amplification (MLPA) and Methylation - specific MLPA. In all the cases of bilateral retinoblastoma we trapped point nonsense mutations. In 5 out of 14 (35%) patients point mutations were detected and 9 out of 14 (65%) were negative. In negative patients (4 out of 9 - 44,4%) we requested samples of tumor tissue, where we managed to detect particular mutations that caused the disease.

Conclusion: Analyzed RB1 gene mutations were compared to cases retrieved from RB1 gene Mutation Database (Lohmann 1999). We also discovered new, yet unpublished mutation cases, in majority of them a deletion occurrence was involved, or nucleotide insertion.

P06.147 Adnexectomy status is the critical feature for association between serum Selenium level and the risk of cancer in BRCA1 carriers

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In these analysis we asked whether serum selenium levels were different in women with a BRCA1 mutation who developed breast or ovarian cancer, compared to matched mutation-positive controls who did not develop cancer. We conducted a nested case-control study of 60 women with breast cancer and 15 women with ovarian cancer (and 75 matched controls) who were diagnosed among a cohort of 518 women in a selenium supplementation trial and for whom a blood sample had been taken for serum selenium measurement. Cases and controls were matched for age at enrolment, past history of breast cancer, oophorectomy and whether they received selenium supplement or placebo during cancer chemoprevention trial. Selenium measurements for this analysis were performed one year after above trial. The mean serum selenium level was 74.4 µg/l for the 75 cases, versus 72.8 µg/l for controls.

Selenium level was the independent risk factor for breast/ovarian cancer risk among BRCA1 carriers stratified depending on oophorectomy. For carriers after adnexectomy the optimal concentration of Selenium in the blood was <70 µg/l (p = 0.033; OR = 0.27; CI = 0.08-0.8).

For carriers without adnexectomy the optimal selenium concentration was 60-80 µg/l. This sub-group have had ~20 fold lower risk of cancers as compared with carriers with selenium blood level <60 µg/l (OR = 18.6; p = 0.024; CI = 0.04 - 0.80) and ~2 fold lower risk of cancer as compared with carriers with selenium level > 80 µg/l (OR = 0.57; p = 0.43; CI = 0.2 - 1.6).

P06.148 Molecular characterisation of SH2D1A and ODZ1 gene deletions in three patients with variable phenotype of X-linked lymphoproliferative syndrome

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The SH2D1A gene encodes an adaptor protein interacting with the cytoplasmic tail of SLAM family members. Mutations of SH2D1A are responsible for the X-linked lymphoproliferative syndrome.

We present 3 patients from 2 families with complete deletions of the SH2D1A gene. The first patient was an 18-year-old man with Burkitt

lymphoma treated by intensive chemotherapy at the age of 16 followed by severe aplastic anaemia treated by stem cell transplantation from his brother. Molecular analysis showed a deletion of all *SH2D1A* exons. His maternal cousin, a 25-year-old man with mild learning difficulties, mild autistic features and severe epidermolysis bullosa-like dermatitis that manifested at the age of 8 years, carried the same deletion. Due to this atypical phenotype, array CGH was performed to specify the extent of the deletion. It was about 65 kb long and removed also the 3' terminal part of the *ODZ1* gene. The molecular defect was identical in both cousins. The third patient was a 20-year-old man who developed EBV-driven lymphoproliferation at the age of 3. He recovered after chemotherapy and now, at the age of 21, he is well, suffering only from hypogammaglobulinaemia. His brother died of lymphoproliferation in infancy. The deletion in this patient spanned over 200 kb, affecting the entire *SH2D1A* and a part of *ODZ1*. FISH analysis was used to identify female carriers in this family. Our study can help to decipher the phenotype contribution of genes deleted together with *SH2D1A* in carriers of large Xq25 deletions.

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P06.149 The different effect of the gene knockdown on apoptosis of two human colon cancer cell lines.

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We investigated the effects of gene silencing mediated by small interfering RNA (siRNA) on two colorectal cancer cell lines: HT-29 and CaCo2. The HT-29 is characterized by APC gene mutation that is absent in CaCo2 cell line. In accordance with this we found an expression of c-Myc gene in HT-29 cells approximately five times more in comparison with CaCo2 cells. The expression c-Myc mRNA was determined also following siRNA transfer to cells. After c-Myc gene silencing we observed apoptosis among HT-29 cells (17%, 3 times more than in control). There were no significant difference of apoptotic cell number in CaCo2 culture with and without c-Myc silencing. The c-Myc silencing after heat-shock treatment of cells (44°C, 40 min) for apoptosis induction lead to increasing of apoptotic cell number in both cell lines. However, silencing by siRNA of HSPA5 gene, the member of heat-shock protein 70 family, result in more effect on apoptosis for both cell lines after heat-shock in comparison with c-Myc silencing. The combined using of both mentioned gene silencing lead to 90% of apoptotic cells for HT-29 and 60% for CaCo2 that is significantly more than after heat-shock alone. The data demonstrate different effect of the same gene silencing in dependence on cancer cell genomic characteristics and significance of genes studied for development cancer treatment, especially for colon tumor with APC gene mutation.

P06.150 A combination of anti-IAP interfering RNAs and chemotherapy induce efficient colon tumor cell apoptosis under drug small doses

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The gene silencing mediated by small interfering RNA (siRNA) was used to increase a chemotherapy effect. The siRNA of 19-22 base pair long and small hairpin RNA (shRNA) produced from expression vector were used for this study. A number of colon tumor cell lines: Caco2, HT-29 and HCT-116 were tested for the cisplatin and oxalyplatin sensitivity. The HT-29 appeared to be more susceptible for oxalyplatin. The p53-negative HT-29 cells seem to escape from p53-dependent apoptosis and anticancer platin based drugs activate caspase cascade. We performed serially repeated RNA silencing of caspase linked inhibitors of apoptosis (IAP). Mixed cocktail of anti-FLIP, IAP-2 shRNAs and anti-IAP-5 (Survivin), HspA5 siRNAs showed twice stronger effect than oxalyplatin alone: 38 and 18% respectively. Combinations of IAP-2/FLIP and IAP-5 with oxalyplatin resulted in 50 to 60% apoptosis compared with 10% of the negative control. In summary, anti-FLIP and IAP-2 together with small dose oxalyplatin demonstrated the same apoptotic death of cells as treated with (3x)-oxalyplatin alone. Our data indicate that anti-IAP siRNA seem to be a prominent tool to increase the effect of standard chemotherapy and significantly reduce its overload for cancer patients.

P06.151 High-risk neuroblastoma without MYCN amplification - characterization of the 11q-deletion tumors reveals a poor prognostic chromosome instability phenotype with later onset

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Analysis of chromosomal aberrations is used to predict clinical prognosis of children with neuroblastoma and to stratify risk-based therapy. MYCN amplification (MNA) alone is incomplete as a poor prognostic factor and 11q status has recently been included in risk classification. We analyzed 170 neuroblastomas using high-density SNP microarrays and describe and compare the high-risk groups defined by MNA (n=37) and 11q-deletion (n=21). Median age at diagnosis was 21 months for the MNA group and 42 months for 11q-deleted, while median survival from diagnosis was 16 months for MNA and 40 months for 11q-deletion. Overall survival was similarly poor, 35% at eight years for both groups. MNA and 11q-deletion were almost mutually exclusive; only one tumor harbored both aberrations. The numbers of segmental aberrations differed significantly; the MNA group had a median of four aberrations, while 11q-deleted group had 12. The high frequency of chromosomal breaks in the 11q-deletion group is suggestive of a chromosomal instability phenotype gene located in 11q, and one such gene, H2AFX, is located in 11q23.3 (within the 11q-deleted region in all tumors). Furthermore, in the groups with segmental aberrations without MNA or 11q-deletion, children with tumors with 17q gain had worse prognosis than those with segmental aberrations without 17q gain, who had a favorable outcome in our material. This study has implications for understanding of neuroblastoma genetics, prognostic assessment and choice of therapy for different risk groups and stresses the use of genome wide microarray analyses in clinical management to evaluate patient diagnosis, risk and treatment.

P06.152 MMR system gene expression profile in sporadic colorectal cancer

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Colorectal carcinoma is the second leading cause of death by cancer in Europe as its incidence increases with life span. As colorectal cancer (CRC) develops over a long period of time, screening is a vital prevention and early detection component. Continuing research to detect new highly sensitive and specific noninvasive CRC biomarkers is essential. The aim of this study was to compare 9 mismatching repair (MMR) genes activation levels in normal, polyp and malignant tissues in order to detect a MMR gene expression pattern in sporadic colorectal malignant pathology. Real-Time PCR with TaqMan (Applied Biosystems) probes specific to ANKRD17, EXO1, MLH1, MLH3, MSH2, MSH3, MSH4, MSH5, MSH6 gene transcripts were used. The general tendency observed is a decreased mRNA level of the MMR genes in tumor samples by comparison with normal tissue, with the exception of EXO1 and MSH5. Of the analyzed genes, ANKRD17 mRNA appears to be the most sensitive target and may have a potential value as an additional marker for the existing multitarget assay panel for colorectal cancer detection.

P06.153 Telomere length evaluation in patients with B-chronic lymphocytic leukemia - correlation with other molecular, cytogenetic and immunophenotypic features

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B-cell chronic lymphocytic leukemia (B-CLL) is a heterogeneous disorder characterized by a highly variable clinical course. Classical staging

systems introduced by Rai and Binet help to predict survival in B-CLL, however they do not distinguish patients who will evolve more aggressive disease from those who will remain indolent. Therefore, other indicators related to the genetics and biology of B-CLL are increasingly used for prognosis and treatment response prediction. Recently, some evidences suggest that short telomeres are associated with poor outcome and telomere length might be of predictive significance. During the years 2007 - 2009, peripheral blood and bone marrow samples of 102 patients with B-CLL (53 male, 49 female, mean age 68 years) were analyzed to ascertain whether telomere shortening was associated with genomic aberrations detected by I-FISH (Abbott Vysis), immunoglobulin variable heavy chain (IgVH) mutational status, CD38 and ZAP-70 expression, and telomerase activity. No difference in telomere length between patients with good and intermediate prognosis according to cytogenetics was found, however, in patients with deletion of ATM/p53 gene and IgH rearrangement short telomeres were proved ($p=0.053$). Association between telomere length and IgVH mutational status, ZAP-70, CD38 expression and telomerase activity was found as significantly shorter telomeres in patients with unmutated IgVH status ($p=0.001$), ZAP-70 positivity ($p=0.001$), CD38 positivity ($p=0.01$) and telomerase positivity ($p=0.01$) were detected. Telomere length in combination with other prognostic parameters completes the risk profile and might serve for better subclassification of B-CLL patients with different outcomes and further treatment. Supported by grants MZOVN2005, MSM0021620808, MSMLC535.

P06.154 *TP53* Gene Polymorphisms in Breast Carcinoma Patients

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Matrix metalloproteinases (MMPs) are potent proteolytic enzymes that are known to play key roles in invasion and metastasis of malignant tumours. Tissue inhibitors of metalloproteinases (TIMPs) are endogenous inhibitors of activated MMPs that contribute to normal functions such as tissue repair after injury and development, as well as to pathologic states such as cardiovascular disease and cancer.

There are four known TIMP genes (*TIMP-1-4*) which have both MMP-dependent and MMP independent effects in many cell types including the breast. *TIMP-2* is normally expressed in breast stromal tissue; however, increased expression has been found in ductal carcinoma *in situ* and in invasive breast carcinomas. *TIMP-2* has been found to stimulate cell growth and inhibit apoptosis in breast cancer cells, as well as to inhibit endothelial cell growth and abrogate angiogenesis. This study was conducted to investigate *TIMP2* gene polymorphisms on breast cancer.

In our study we investigated the *TIMP2* G418A and C303T polymorphisms in 42 breast cancer patients and 41 fibroadenoma samples that were used as a control group. DNA was extracted from paraffin embedded tissue sections according to standard protocols. Polymerase chain reaction-restriction fragment length polymorphism was used for the detection of *TIMP2* G418A and C303T polymorphisms.

According to our results *TIMP2* gene polymorphisms don't seem to be associated with breast cancer susceptibility and clinical parameters. However to confirm our results, further studies need to be performed in a larger group of patients and additional polymorphisms of *TIMP2* gene.

P06.155 Human papilloma virus and *TP53* gene mutation in oral tongue SCC and their correlation with tumor characteristics in Iranian population; a multicenter study

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Mashhad University of Medical Sciences, Mashhad, Islamic Republic of Iran. Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, the most common site of oral-cancer development is the anterior two thirds of tongue. *TP53* has been frequently reported to be associated with HNSCC among smokers and drinkers. In recent years, number of patients at younger ages who are non-smokers and non-drinkers but suffer from oral cavity SCC (OSCC) is increasing. For these patients, other etiologic factors such as infection with Human Papilloma Virus (HPV) may be considered. The purpose of this study was to investigate the prevalence of HPV infection and *TP53* mutation in patients with squamous cell carcinoma (SCC) of the tongue and subsequently its significance on cervical lymph node metastases and tumor differentiation. Sections of tissue blocks from ninety five tongue SCC patients, were enrolled. Immunohistochemical (IHC) technique was used to study tissue *TP53* mutation. Polymerase chain reaction (PCR) was performed for detection of high risk HPV types, sixteen and eighteen. Frequency of HPV16 and HPV18 infection were 10.6% and 16% respectively. *TP53* mutation was found in 70.2% of patients. Young patients (aged below 45 years) comprised 20 % of all patients. There was not a significant association between *TP53*, HPV16 or HPV18 presence and higher stages of the tumor, tumor differentiation or presence of nodal metastasis. Although association between HNSCC and HPV infection is being recognized and reported, our data implicates that HPV infection or *TP53* mutation may not play a significant role in oral tongue SCC pathogenesis, differentiation or metastasis.

P06.156 Novel *TP53* germ-line mutations in a Czech families with Li-Fraumeni syndrome

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Li-Fraumeni syndrome is a rare autosomal dominant syndrome characterized by the occurrence of diverse mesenchymal and epithelial neoplasms at multiple sites.

Here we demonstrate 2 novel germ-line mutations detected in Czech LFS families.

A novel large germ-line deletion of *TP53* gene spanning exons 2-11 (confirmed by CGH-array) was identified by MLPA in typical LFS family with early childhood brain tumor and severe family history of bilateral breast cancer cases, liposarcoma and melanoma in 4 generations diagnosed in early adulthood.

TP53 missense substitution p.Ile254Val located in DNA binding domain was detected in a family with 3 relatives with breast, bilateral breast and brain tumors (diagnosed at age of 42-56). This missense substitution has not been yet described as a germ-line mutation but was reported as a very rare somatic mutation in IARC *TP53* database. By the assessment of transactivation capacities measured by Kato et al. p.Ile254Val was designed as functional in IARC *TP53* database. We evaluated a functional status of p.Ile254Val using FASAY, which resulted in loss of function as fully inactivating mutation.

TP53 missense mutants located in the DNA binding domain could be classified as partial deficiency alleles or severe deficiency alleles based on their ability to transactivate target sequences. Several studies indicated that specific mutation in *TP53* may affect the type of tumor and the age of onset but the effect of the functional heterogeneity of the *TP53* mutations on the severity of associated disease has not been assessed.

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P06.157 The role of pregnancy on hemangioblastomas in von Hippel-Lindau disease: a retrospective French study.

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Von Hippel-Lindau disease (VHL) [OMIM 193300] is a dominantly inherited disorder predisposing to highly vascularized tumors, mainly hemangioblastomas of the central nervous system (CNS) and retina, but also renal cell carcinomas, pheochromocytomas, pancreatic neuroendocrine tumors and endolymphatic sac tumors. The disease results from germline mutations in the *VHL* tumor-suppressor gene that plays a key role in response to hypoxia and angiogenesis.

A potential growth of hemangioblastomas during pregnancy with consequent risk to the mother and fetus has been reported in a few case reports and an only study in a small population of VHL patients. Thus, we performed a retrospective and comparative French study in 269 women from 172 families from the national VHL clinical database. Patients were divided into two groups according to their gestational status. The study focused on the occurrence of new hemangioblastomas and complications of previously known tumors. Available data of imaging follow-up of CNS and retina were collected in 176 women with at least one pregnancy (group 1) and 93 women with none (group 2). The results showed more complications of hemangioblastomas in group 1 ($p=0.031$) with increased symptoms and the need for surgical treatment in emergency in some cases.

To our knowledge, this is the first study analysing the pregnancy's effect in a very large series of VHL patients. This work underlies the necessity of a close follow-up during pregnancy and a systematic CNS Magnetic Resonance Imaging without injection is recommended during the fourth month of pregnancy in women with hemangioblastomas.

P06.158 Identification of germline mutation in a Turkish patient with Von Hippel-Lindau disease

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Von Hippel-Lindau (VHL) disease is an uncommon, dominantly inherited multisystemic cancer syndrome. Various benign and malignant tumors appear in multiple organs including eye, kidney, pancreas, broad ligament, epididymis, adrenal gland and central nervous system in the disease. Retinal capillary hemangioblastoma is hallmark tumor lesion in the eye. VHL disease caused by germline mutations in the VHL tumor-suppressor gene located on chromosome 3p25-26. Germline mutations in the VHL gene span three exons.

In this study, germline VHL mutation identified in a Turkish patient affected with the disease. Mutation screening of three exons in VHL gene was performed to detect disease causing pathologic allele. Mutation analysis in exon 3 of VHL gene revealed that there is a disease causing heterozygous R161X mutation in the patient. Different type of VHL mutations in literature were classified and VHL truncating mutations were found to be associated with hemangioblastomas and renal cell carcinoma.

Analysis of germline mutations in VHL gene and correlation between mutations and ocular phenotype may light on understanding of pathogenetic mechanisms underlying VHL tumorigenesis in the eye.

P07 Cancer cytogenetics

P07.01 Rare cytogenetic findings in two cases of acute leukemia

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Identification of recurring chromosomal abnormalities has a major impact on risk evaluation and for treatment of the patients with acute leukemia. Cytogenetic and molecular genetic analyses of childhood acute lymphoblastic leukemia (ALL) are today used routinely for diagnostic and prognostic evaluation; chromosomal aberrations are revealed in approximately 70% of patients. Genetic alterations can be detected in

approximately 80% of acute myeloid leukemia (AML), these alterations are considered important in initiating events in the process of leukemogenesis and as indicators of clinical outcome. In the cytogenetic laboratory at the University of Medicine and Pharmacy Timisoara we have investigated 56 cases of acute leukemia, 26 cases of ALL and 30 cases of AML. In one case of ALL, the cytogenetic findings were trisomy 4 associated with trisomy 17. Trisomy 4, a numerical abnormality, is commonly associated with high hyperdiploidy and is very rarely found in cases of ALL with low hyperdiploidy. The functional consequences of hyperdiploidy are not clearly understood. It remains to elucidate if the extra copies of particular chromosomes found in hyperdiploid ALL lead to increased expression or to more marked changes in expression of particular genes. In one case of AML the only cytogenetic aberration found was trisomy 4. Trisomy 4 is a rare cytogenetic abnormality in patients with AML, with a frequency of less than 1%. The prognostic significance of this abnormality in patients with AML is not clear, but appears to be poor. More data are needed for understand the prognostic significance of such rare karyotypic abnormalities.

P07.02 Cytogenetic analyses of the chromosome 11 duplication/amplification in acute myeloid leukemia

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Gene amplification is a frequent genetic abnormality in solid tumors, whereas it has been rarely detected in hematological malignancies. Activation of proto-oncogenes is associated with aggressive growth of tumor cells and poor prognosis. In acute myeloid leukemia (AML), repeated target of gene amplification is chromosome 11, particularly band 11q23.

During years 2006 to 2009, bone marrow cells of all newly diagnosed patients with AML were examined by conventional cytogenetics and FISH with a Vysis MLL Break Apart Rearrangement probe (11q23.3). In some cases, target regions of chromosome 11 amplification were investigated by Vysis LSI ATM (11q22.3), LSI CCND1 (11q13.2) probes (Abbott) and/or by methods mFISH/mBAND 11 (MetaSystems).

Chromosome 11 aberrations were proved in 32 of 148 patients: rearrangements in 28, duplication/amplifications in 13 and deletion in 3 patients. The amplification was presented as: amplification of the 5' MLL (myeloid/lymphoid leukemia) gene (1x), trisomy 11 (3x), partial trisomy 11q (5x), isochromosome 11q (1x) and multiple amplification (3x).

In conclusion, chromosome 11 duplication/amplification has been proved to be present in approximately 10% of AML cases. Amplicons vary in size and can be located on both arms of chromosome 11. However, overrepresentations usually include a band 11q23 and MLL gene. The identification of true target genes of amplification is important for understanding the cancer pathogenesis and can be useful for cancer therapy selection. Therefore, a combination of conventional and molecular cytogenetic methods is needed for their detection.

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P07.03 Novel translocation t(6;17)(p12;p11.2) in de novo acute myeloid leukemia with complex karyotype - a case report

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50-70% of acute myeloid leukaemia (AML) patients have cytogenetic abnormalities and 10-15% of adult AML show complex karyotypes. It is well established that karyotype represent a key feature for diagnosis and prognosis.

We present a *de novo* AML case (72 year-old, female) with complex chromosomal abnormalities identified at diagnosis, including a novel translocation t(6;17)(p12;p11.2).

Bone marrow chromosomal studies were performed on GTG-banded slides. FISH with painting probes (chromosome 9 - Kreatech, chromosome 6 - Cytocell), locus specific probes (TP53 - Vysis, subtelomeric 17pter Kreatech, dual fusion BCR/ABL probes - Vysis) and BAC FISH probes (RP11-525O11 - 17p11.2, RP11-129E3 - 17q24) were applied

for molecular characterization.

Two malignant clones were detected, one bearing abnormalities of chromosomes 6 and 17, and the other having additional abnormalities - partial deletions of both short and long arm of chromosome 9. Both homologous chromosomes 17 were involved, in different anomalies: a t(6;17)(p12;p11.2) generating der(17)(6;17)(p12;p11.2)(wcp6+, ST17pter-TP53-,RP11-525O11+,RP11-129E3+), and an interstitial deletion, not encompassing TP53, del(17)(p11.2p1?3)(STpter+,TP53+,RP11-525O11-,RP11-129E3+). The anomalies involving chromosome 9 are still to be elucidated. Painting FISH suggests a deletion, as no chromosome 9 material is observed elsewhere. ABL region is not deleted: der(9)del(9)(p?)del(9)(q?q34)(wcp9+,ABL+).

The pathogenesis of AML with complex karyotype is less well understood when compared with AML associated with specific genetic defects. Thus, unravelling new cytogenetic abnormalities in AML with complex karyotype might bring some important insights in understanding this heterogeneous disease.

To our knowledge, this is the first reported case with a translocation t(6;17)(p12;p11.2) as part of a complex karyotype in *de novo* AML.

P07.04** Chromosomal alterations evaluated by aCGH in patients presenting pediatric cancer and congenital dysmorphisms

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Oncogenesis is a multistep process that, generally, requires several years until manifestation of the disease. Accordingly, childhood neoplasias must be rare. It seems reasonable to assume that the premature appearance of neoplasias often involves genetic alterations constitutively present in somatic lineages of the patients, shortening the period of time necessary to trigger the tumorigenesis. Furthermore, an increased frequency of congenital defects has already been reported among pediatric cancer patients. The presence of variation in copy number of DNA segments (copy number variations - CNVs) affecting gene(s)-dosage could explain the concomitant occurrence of cancer and associated phenotypes. Currently, array-based comparative genomic hybridization (aCGH) is a useful tool to detect submicroscopic deletions or duplications. In this work, we used a 180K aCGH platform (Agilent) to investigate the presence of such chromosomal alterations in patients presenting pediatric cancer associated with clinically minor anomalies or congenital defects, an approach almost unexploited to identify genes involved in cancer and/or associated phenotypes. Among 31 patients evaluated so far, including one XY female, seven of them (22.6%) presented submicroscopic chromosome alterations never or rarely reported in DGV (Database of Genomic Variants), being four microduplications (at 4q13.3, 11p15.5, Xq27.2 and 18q21.33) and three microdeletions (at 7q36.1, 5p13.1 and 3p14.2). Our initial results point to chromosomal regions possibly involved with the emergence of childhood cancer. Financial support: FAPESP.

P07.05 Cytogenetic characteristics of basal cell carcinoma

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Basal cell carcinoma is the most common epithelial cancer in humans. Known karyotypic abnormalities in this tumor are nonrandom, rarely including evidence of clonal evolution. The aim of this research was to investigate the frequencies of numerical and structural chromosome aberrations in primary cultures of basal cell carcinoma. Cytogenetic analysis was conducted over 10 successfully harvested samples. Observed chromosome aberrations were scored according to the International System for Human Cytogenetic Nomenclature. Aneuploidy was the most frequent among observed numerical chromosome aberrations. Aneuploid cells were found as it was expected because aneuploidy is probably the only mutation capable to clarify all aspects of carcinogenesis.

P07.06 Predictive genetic diagnosis of breast cancer: detection of large rearrangements in the genes BRCA1 and BRCA2 by Molecular Combing

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The *BRCA1* and *BRCA2* genes are involved with high penetrance in breast and ovarian cancer susceptibility. Among patients with breast cancer and severe family histories of cancer who test negative for *BRCA1* and *BRCA2* point mutations, about 10 to 15 % can be expected to carry large genomic alterations (deletion or duplication) in one of the two genes, mostly on *BRCA1*. Large rearrangements are missed by direct sequencing. Thus, effective methods to identify these types of mutation should be integrated in current diagnostic procedure to obtain a more comprehensive genetic screening strategy. Molecular Combing is a powerful FISH-based technique for the direct visualization of single DNA molecules which allows exploration of the entire genome at high resolution in a single analysis. We have developed a novel predictive genetic test based on molecular combing, for which specific *BRCA1* and *BRCA2* "genomic morse codes" and associated analysis platform have been designed and validated on 10 breast cancer patients with severe family history. Large rearrangements corresponding to deletions and duplications of one to several exons and with sizes ranging from 3 kb to 40 kb have been detected on both genes. Importantly, the mutations identified by Molecular Combing confirm the results previously obtained by high-resolution zoom-in aCGH (11k) on the same patients, with a resolution in the 1 - 2 kb range.

P07.07 Molecular cytogenetic analysis of novel recurrent abnormality of chromosome 7 in three pediatric patients with myelodysplastic syndrome (MDS)

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Abnormalities of chromosome 7 are the most common cytogenetic findings in childhood MDS occurring in ~55% of children with primary disease. Chromosome 7 aberrations are associated with poor outcome and most frequently occurs as a loss of whole chromosome or interstitial deletion of long arm with major breakpoints at 7q22 and 7q35-q36. Deletions of short arm are less frequent.

We present an unusual structural abnormality leading to loss of whole short and long arm of chromosome 7 which was found in bone marrow cells of three children with MDS. For precise characterization of the marker chromosome conventional cytogenetics and FISH analyses using Vysis CEP 7/LSI 7q31, ToTelVysis probe mix (Abbott Molecular) and XCye 7 color kit (MetaSystems) were performed. A tiny "dot-like" marker chromosome was shown to be composed solely of chromosome 7 centromeric region and was described as der(7)del(7)(p11)del(7)(q11) in all three cases and was presented in 79%, 53% and 15% of screened nuclei, respectively.

Together with one previously published case¹ it represents a new recurrent karyotypic abnormality involving chromosome 7 in childhood MDS. Whether this novel chromosomal abnormality is a new cytogenetic entity or a specific prognostic subgroup must be evaluated on larger series of patients. Cytogenetically, reported marker, due to its size, can easily be overlooked by G-banding analysis. Therefore all cases with an apparent monosomy 7 observed by conventional cytogenetics must be interpreted with caution and should be confirmed by molecular cytogenetic methods.

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¹Zamecnikova A. Cancer Genet Cytogenet 2008;180:163-4.

P07.08 Isochromosome i(9)(q10) in childhood T-cell acute lymphoblastic leukemia

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Isochromosome i(9)(q10) is a rare recurrent chromosomal aberration described in bone marrow cells of ~1% children with acute lymphoblastic leukemia (ALL). This abnormality more often occurs in patients with pre-B immunophenotype, although rare cases of T-ALL have also been reported.

We examined 62 newly diagnosed children with T-ALL by combination of cytogenetic and molecular cytogenetic methods. In two of them, i(9)(q10) was found, in one case as sole aberration and in the other combined with trisomy 8 and t(10;14)(q24;q11). Findings were confirmed by mBAND and mFISH (XCyte 9, 24XCYte, MetaSystems). Chemotherapy was administered to both patients (BFM Interim) and they are living in complete remission 10 and 6 months after diagnosis.

The biological effect of i(9)(q10) is not well established. Formation of isochromosome results in partial monosomy of the short arm and partial trisomy of the long arm of chromosome 9. These two pathogenetic events - overexpression of several proto-oncogenes (including the *ABL* gene at 9q34) and deletion of various tumor-suppressor genes (including *CDKN2A* at 9p21.3 or *PAX5* at 9p13), have been suggested to play significant role in malignant proliferation of leukemic cells. Specifically, loss of the known candidate gene *CDKN2A* leading to deregulation of cell cycle, is one of the most important deletions in childhood T-ALL. Nevertheless, the i(9)(q10) is a scarce genomic abnormality and therefore, its clinical implication is extremely hard to evaluate. Thus, the significance of i(9)(q10) in pathogenesis of T-ALL needs to be further studied on larger cohorts of patients.

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P07.09

Recurrent chromosomal unbalance translocation in two cases of chronic lymphocytic leukemia with TP53 deletion.

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Chronic lymphocytic leukaemia (CLL) is the most common leukaemia in the Western world. The analysis of chromosomal aberrations has provided significant prognostic information. The use of metaphase cytogenetics, however, has turned out to be problematic, due to the low mitotic index of most CLL cells even in the presence of B-cell mitogens. The analysis of aberrant chromosomal regions by fluorescence in situ hybridization (FISH), which can be applied to interphase cells, resulted in the detection of clonal aberrations in more than 80% of CLL patients. Deletion of the locus of the TP53 gene identifies the most aggressive subset.

We present here two patients with TP53 deletion and a recurrent translocation that involve chromosomes 8 and 17.

Conventional cytogenetic studies were carried out with G-banded according to standard protocols. FISH analyses were performed in cell suspension from the conventional cytogenetics culture, with CLL kit LSIP53 / LSI ATM and LSI D13S319 / LSI 13q34 / CEP 12 (Vysis). Two hundred nuclei were analysed for each probe.

Both patients showed -8 and der(17)t(8;17)(q12;p13) and TP53 deletion.

From our knowledge it is the first time that it recurrent chromosomal abnormality is described. We can remark the relevance to combine conventional and molecular cytogenetics in CLL to detect new recurrent clonal chromosomal aberrations that can help us to understanding the biological mechanisms related with the pathology and evolution of CLL patients.

P07.10 Chronic lymphocytic leukemia with trisomy 12 associated with t(14;18) or trisomy 18.

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Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder. This is the most common leukemia of the adult population in Western countries. The chromosomal abnormalities associated with CLL are: trisomy 12, del(13)(q14), del(11)(q22-23), del(17)(p13) and del(6)(q21). Some studies have reported the presence of trisomy 12 associated with other chromosomal abnormalities as t(14;18)(q32;q21)

and trisomy 18. The t(14;18)(q32;q21) has been identified in 90% of follicular lymphomas and 20-30% of diffuse large B-cell lymphoma, but is rare in CLL. The CD10 marker is expressed in of follicular lymphoma and is not expressed in CLL. The expression of ZAP70 and CD38 markers are both associated with a poor prognosis in CLL. The aim of this study was to describe the clinical and pathological features of 5 CLL patients with trisomy 12 associated with t(14;18)(q32;q21) or trisomy 18. Material and Methods. In this study, three patients showed by karyotype and/or FISH studies trisomy 12 + t(14;18)(q32;q21) and two patients trisomy 12 + trisomy 18. The CLL markers were studied by flow cytometry in 4 cases. CD10 was studied in three cases [+12, t(14;18)(q32;q21) two cases; +12,+18 one case]. Results. In all cases the ZAP70 marker was negative and CD38 was positive. CD10 marker was negative in all cases. Follow-up was available in two patients (12, + t(14;18)(q32;q21); +12 + 18) and none of them progressed. Remarks. CD38 marker was positive and ZAP70 negative in all the CLL patients included in the study. The lack of expression of CD10 marker expression excludes the follicular lymphoma diagnosis.

P07.11 Complex karyotype in a case of CMML

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The chronic myelomonocytic leukemia (CMML) is a haematological disease of difficult classification because of their clinical characteristics and their evolution. Initially, according to FAB classification, the CMML was considered a myelodysplastic syndrome. Recently, CMML is included in a new category: the disease myelodysplastic / myeloproliferative according to WHO classification.

The cytogenetic analysis may be important in the characterization of disease and prognosis. Approximately 20 to 40% of patients with CMML have chromosomal abnormalities, but none is specific to the disease. The most common aneuploidies involve the chromosomes 8 and 7, structure abnormalities in chromosome 12 and complex karyotypes are also described.

The authors present the results of a cytogenetic study in a patient with CMML, with 10 years of development, clinical and analytical stable, despite leukocytes sustained over 20.000/ μ l.

Bone marrow cell cultures were made and all the technical procedure to obtain metaphases was performed according to the protocols in the laboratory.

The karyotype was: 46, XY, der(1)t(1;5)(q44;q31), der(5)del(5)(q14q31), t(15;22) confirmed by FISH technique. This is a complex karyotype, unbalanced, presenting two translocations in all metaphases analyzed.

The authors present a bibliographic revue.

P07.12

Instability at common fragile sites as a possible cause of recurrent chromosomal rearrangements in colorectal cancer

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Colorectal cancer (CRC) ranks among the leading causes of cancer death worldwide. The identification of genes that influence colorectal cancer susceptibility and progression therefore remains an essential goal of basic and clinical research. Specific patterns of structural chromosomal aberrations have been associated with this tumour type. However, despite these findings, the molecular events underlying cancer susceptibility and chromosomal instability in these tumours are not well understood.

Early-stage colorectal cancer development is associated with DNA replication stress inducing DNA double-strand breaks and consequently genomic instability. Such genomic DNA damage, represented mainly by deletions and amplifications, occurs particularly at specific loci that are prone to DNA double-strand breakage. These loci are present in all human individuals and are referred to as common fragile sites (cFSs). Rearrangements within cFSs were identified using customised CGH arrays with spotted sequences of 21 known cFSs. Aberrations were detected within multiple cFSs in all samples investigated, suggesting that cFSs may well be involved in colorectal tumourigenesis. Breaks seem to most prominently occur within fragile sites *FRA3B* (76.5%) and *FRA16D* (47%), usually within the regions spanning *FHIT* and *WWOX*, respectively. Additionally, aCGH data indicate several other active cFSs, such as *FRA1A* (41.2%), *FRA2C* (41.2%) and *FRA2E*

(35.3%). Many of the observed rearrangements seem to affect cFS genes, making them potential candidates for further study. Investigating cFS loci for genomic alterations in cancer cells, rather than the entire genome, is likely to accelerate the search for CRC candidate genes as biomarkers for clinical patient management including targets for therapies.

P07.13 Karyotype and first complete remission rate in adult acute lymphoblastic leukemia

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The associations of cytogenetics with complete remission (CR) rates, time to achieve first CR and therapy resistance were studied in 35 untreated adult ALL patients in a clinical trial comparing 3 patient's prognostic groups: poor risk, standard risk and miscellaneous. Chromosomal analyses of bone marrow were performed by G-banding and aberrant chromosomes were examined using fluorescence in situ hybridization (FISH) with locus specific and centromere probes (Vysis). The statistical variables tested for potential prognostic value were: molecular-cytogenetic abnormalities, age, WBC, immunologic subgroup, CR, frequency of resistant disease and early deaths in ALL patients. Clonal chromosomal abnormalities were detected in 18 of 35 (51.4%) adult patients with ALL with the follow separation: poor risk group (t(9;22), t(11q23), t(8q24) and complex karyotype)- 31.4%, standard risk group (normal karyotype, t(1;19) and hyperdiploidy)- 65.7% and miscellaneous risk group (del(12p), i(7q)and del(4p)) - 14.3%. CR was achieved in 71.4% of patients and resistant disease (RD) or early deaths were detected in 34.4% of cases. Although the presence of poor pretreatment laboratory characteristics, the group with miscellaneous abnormalities is suggestive of having better CR rate compared with the poor risk and standard risk groups (83.3% vs. 68.7% and 76.5% respectively). Patients with t(9;22), t(8q24) and complex karyotype had higher rate of RD (50-75%) then patients with other aberrations or normal karyotype. Our study emphasizes that karyotype is an independent prognostic factor in adult ALL after intensified treatment regimens. In multivariate analysis, karyotype retained its prognostic significance for CR rate, time to achieved remission and RD.

P07.14 Conventional Cytogenetics in Acute and Chronic Leukemias: A Laboratory Report

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Cancer is, in general, more common in industrialized nations, but there has been a growth in cancer rates in developing countries, particularly as these nations adopt new diet and lifestyle habits. The burden of leukemias is increasing by the day and the incidence of different types of leukemia varies with the age in different parts of India.

We received a total of 85 Bone Marrow Aspirates over a period of 10 months between November 2008 and September 2009. Conventional GTG chromosomal analysis was carried out according to the modified method of Morehead. 30 metaphases were analyzed and 10 karyotypes made.

The 85 cases included 49 (57.6%) males and 36 (42.4%) females. Of these, normal karyotype was revealed in 49/85 (57.6%) while an abnormal karyotype was seen in 36/85 (42.4%) cases. Karyotype with t(9;22) was seen in 10/36 (27.8%), t(8;21) in 5/36 (13.9%) and t(15;17) in 6/36 (16.7%). Other abnormalities i.e., 13q-, inv (3), monosomy 7, 5q+, t(5;11), der7, dup (1p), trisomy 8, t(1;3), 2x (del) 22? and aneuploidy were seen in 15/36 (41.7%) cases. Our results show that conventional cytogenetics could be used as first line diagnostic tool for different types of leukemia. Further it gives a complete picture of the chromosomal changes compared to FISH or Real time PCR which are specific. Conventional cytogenetics can also be used to differentiate between acquired and constitutional changes and can also detect treatment induced random aberrations. It is also cost-effective in comparison to FISH and Real Time.

P07.15 Cytogenetic abnormalities del(17p) and del(11q) in chronic lymphocytic leukemia

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Background. In chronic lymphocytic leukemia (CLL), the presence of del(17p) is associated to chemotherapy resistance and poor prognosis. Del (11q) also confer an unfavorable outcome. **Aims.** To asses the incidence of del (17p)/del (11q) in CLL patients in different phases of their disease and their impact on treatment requirement. **Methods.** From September 2000 to december 2009, 87 CLL patients were included in this study. There were three groups: 45 cases at diagnosis, 24 at first progression and 18 at progression after one or more therapies. Cytogenetic abnormalities and CD38 expression were analyzed. **Results.** The incidence of del(17p) was 22%, del(11q) was 11% and CD38 expression in 9% of the cases. All patients CD38+ had del(17p) or del(11q). Cases with del(17p) or del(11q) had a treatment-free interval that was on average half of that of cases without these abnormalities (23 vs 40 months) as well as patients with CD38 pos vs neg (18 vs 42 months). **Conclusions.** Del(17p) and del (11q) occur in CLL cases associated with CD38 expression and show a rapidly progressive course of the disease.

P07.16 Using the FISH method for an identification of genetic alterations in cervical carcinoma and cervical intraepithelial neoplasia: gain of human telomerase gene (hTERC)

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Cervical cancer is the second most common cancer in women in the Czech Republic, approximately 400 women die of this disease every year. It is known that this carcinoma develops from precancerous cervical intraepithelial neoplasia (CIN) which is characterized by series of genetic abnormalities. Gain of *hTERC* (3q26) was the most consistent chromosomal abnormality found in cervical carcinoma. It was discovered that gain of *hTERC* gene is associated with progression from precancers (CIN1, CIN2, CIN3) to cervical carcinoma.

In our work, we studied copy number changes of the chromosomal region of *hTERC* gene using the FISH method in cytological smears obtained from 30 patients of the Departement of Gynaecological Oncology of Masaryk Memorial Cancer Institute Brno. Gain of *hTERC* gene was found in 16 from 30 (53 %) patients with the diagnosis of cervical cancer or with CIN1-CIN3. It was confirmed that gain of *hTERC* is significantly associated with cervical carcinoma.

In 2009 a new DNA probe for simultaneous identification of HPV infection and examination of copy number changes of *hTERC* and *MYCC* genes using the FISH method was created by Abbott. The initial experiences with detection of *hTERC* and *MYCC* genes in HPV positive cells in CIN3 are presented.

The results of genetic analyses should serve as a supporting tool for the determination of samples containing high risk HPV infections and chromosomal aberrations linked to the potential progression and tumor proliferation.

P07.17 Assessment of HER-2 gene amplification in breast cancer

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A fraction of breast cancers, as part of their development, undergo gene amplification. Amplification and overexpression of *HER-2* oncogene seems to remain stable over the course of disease. Gene overexpression is an important marker of poor prognosis and a useful determinant of susceptibility to trastuzumab. The present study aimed to evaluate *HER-2* amplification/overexpression status in breast cancer patients by FISH technique and immunohistochemistry. The study was performed on 50 selected cases with primary mammary carcinoma. We used immunohistochemistry for *HER-2* oncoprotein and FISH as a follow up test for ambiguous results. From the 50 tumors, 34% presented different degrees of positivity with immunohistochemistry; 66% did

not express the oncoprotein c-erbB2. Cases with 2+ immunostaining score were interpreted as uncertain and *HER-2* status was determined by FISH to reveal genic amplification. 3 cases showed gene amplification. *HER-2/CEP17* ratio was determined using the rate between *HER-2/neu* signals and *CEP17* signals in 20 nuclei. We could appreciate amplification in 4 cases, 2 with „low level“ amplification and 2 with „high level“ amplification. Polyploidy was considered in cases with at least 3.0 signals from chromosome 17. We identified 1 case of polyploidy. *HER-2* amplification/overexpression are used as prognostic markers, but also as predictive markers for breast cancers. Thus, in positive cases, tumors are faster growing, more aggressive and less responsive to chemotherapy and hormone therapy. Identification of *HER-2* - positive tumors is certainly crucial in order to identify patient candidates for anti-*HER-2* therapies.

P07.18 Chromosome aberrations in hypertrophic gastritis

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Gastric cancer takes a special place among human tumor pathology. In most cases this type of neoplasia arises on the background of continuously existing changes of mucosa. Precancerous lesions are usually presented by morphological damages of epithelium and replacement of normal mucosa by dysplastic tissue. However, oncogenic potential of these changes as well as underlined genetic mechanisms remain unclear. One of the possible reasons of tumour transformation is accumulation of multiple chromosome aberrations. But their prevalence among different types of preneoplastic lesions is not fully investigated. The aim of present research was molecular cytogenetic analysis of hypertrophic gastritis tissues. DNA from 15 samples was investigated by CGH. The most common chromosome aberrations were gains of chromosome regions 1q32-q42 (93.3%), 10q24 (73.3%), 15q22 (66.3%) and losses at 9p13 (66.3%), 9q21 (60%) and 1q21 (60%). Our data indicate that these aberrations can be early events in gastric carcinogenesis. It is possible, that cells with observed abnormalities can have selective advantages and form foci of gastric neoplasia. This study was supported by Federal Agency of Education (P-1706).

P07.19 Making of JAK2 RG kit with Real Time PCR method for the First Time in Iran

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JAK2 (Janus Kinase 2) is a Tyrosin Kinase located in cytoplasm with essential role in signaling pathways for cytokines and growth factors. The acquired mutation G1849T leads to substitution of valine with phenylalanine (V617F). This substitution results in constitutively active JAK2 which leads to uncontrolled cell proliferation in the absence of growth factors. This mutation is found in the majority of BCR-ABL-negative myeloproliferative disorders (MPDs) and has become a main diagnostic test for polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF).

JAK2 RG kit is intended for use with Rotor Gene 6000 and for the detection of JAK2 (V617F) mutation in genomic DNA extracted from human cells. This kit is intended for research use only. The analytical detection limit of the kit was assessed with serial dilution series of the cloned target in wild type genomic DNA and showed a limit of detection equal to 0.01% (1:10,000). This sensitivity is achieved only if 5 to 10 million WBC have been extracted and eluted in 200ul which is equal to roughly 100,000 genomes per reaction.

P07.20 Evaluation by standard cytogenetic and FISH of non treated patients with LLC-B

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The chronic lymphocytic leukemia (CLL) is the most common form of leukemia in Western countries. The leukemia of B lymphocytes constitutes about 95% of all cases of CLL. The cytogenetic analysis in the study of B-CLL is important, allowing detecting chromosomal changes associated with this type of leukemia, namely, the trisomy of chromosome 12, deletions in *loci* located on chromosomes 13q14, 11q22-q23,

17p13 and 6q21, with prognostic implications and sometimes therapeutics.

The authors present a study consisting in the analysis by cytogenetic and fluorescent *in situ* Hybridization (FISH) of 25 blood samples of patients with B-CLL, before any treatment.

For each sample were made two kinds of culture, with different mitogens (pokeweed and TPA). All the technical procedure to obtain metaphases was performed according to protocols optimized in the laboratory.

GTL bands were applied and FISH technique with the probes for the unique sequence of chromosomes 13 and 17, and α -satellite probe for chromosome 12 were performed.

The authors present the analytical and clinical data of 25 patients and results of cytogenetic and FISH. A bibliographic revue is presented.

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P07.21** The role of microRNAs in chromosome instability driven by telomere shortening in human prostate cancer cells.

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Chromosomal instability appearing early in tumor progression is a hallmark of almost all cancers. The majority of tumors exhibit abnormal karyotypes consisting chromosomal breaks, fusions and translocations. Recent studies demonstrated that microRNAs are located in chromosomally instable regions. Previously, we have compared karyotypes of commonly used prostate cancer cell lines including DU145, PC3, LNCaP and an immortalized prostate cell line PNT1a as the presence of common regions of genomic instability. Thirty three microRNAs including miR-579, miR-580, miR-1274a, miR-581, miR-449a, miR-449b, miR-1974, miR-583, miR-1244, miR-584, miR-143, miR-145, miR-378 were located on chromosome 5 carrying one of the most consistent regions of instability. Prostate cancer cell lines and PNT1a cells have been transfected by one of these microRNAs, miR145. PNT1a cells have also been transfected by anti-miR-145. Chromosomal preparations were analyzed for any break, aberrations or telomeric associations. Cells transfected by miR-145 showed increased number of telomeric associations and clumping. These preparations had less number of cells in mitosis as well. Our data suggest that microRNAs might play a role, in chromosomal instability driven by telomere shortening.

This study is supported by a grant (18S051) from The Scientific and Technological Research Council of Turkey.

P07.22 Oligonucleotide array-based comparative genomic hybridization analyses of gain 1q21 in multiple myeloma

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Multiple myeloma (MM) is the second most common hematological malignancy. It is characterized by malignant transformation and clonal proliferation of B-lymphocytes and their accumulation in bone marrow. MM is incurable, although usage of the new drugs significantly increased survival of patients in last years. MM is characterized by presence of specific genetic changes present in plasma cells. One of our fields of interest is to analyze gain on long arm of chromosome 1, which is currently one of the most studied chromosomal changes in MM.

Using oligonucleotide array-CGH we analyzed 41 MM samples with gain of 1q21 (CKS1B gene) detected previously by FISH. As input material for array-CGH we used DNA from CD138+ cells separated from bone marrow using magnetic or fluorescence activated cell separation.

Taking together 76% (13 of 41) cases had gained not only 1q21 locus but whole 1q arm. Moreover, another 17% (7 of 41) had at least 100 MB gain anywhere at the 1q arm. It is obvious, that not only CKS1B gene,

but other genes are affected as well, for example IL-6R or BCL6. Many of them are possibly negative prognostic factors in MM. According to the FISH data (in about 95% cases we detect max. two extra copies of CKS1B), we did not find any high-level amplification of 1q21. In all cases, 1q aberrations were associated with additional copy number alterations, including monosomy of chromosome 13 or hyperdiploidy. Supported by grants and projects MSM 0021622434 and 0021622415, IGA NS10207-3/2009 and 10406-3, and LC 06027.

P07.23 t(1;8)(q32;q24): a novel cytogenetic abnormality in pediatric acute lymphoblastic leukemia (ALL)

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MYC, one of the first identified oncogenes, codes for a transcription factor commonly deregulated in tumorigenesis. Chromosomal translocations joining the immunoglobulin (IG) and MYC genes have been extensively reported in Burkitt and non-Burkitt lymphomas, but data concerning MYC rearrangements with non-IG partners are few. We describe a new translocation involving MYC in pediatric ALL. An 11-month-old girl came under our observation with abdominal mass and skin nodules. Bone marrow (BM) aspirate showed 91% lymphoblast FAB L1 morphology. The immunophenotype was positive for CD19, CD20, CyIgM, DR, and negative for CD10 and TdT. Histology of skin nodules revealed a neoplastic infiltrate of Burkitt-like lymphoma, positive for CD19, CD20, DR, λ light chains and negative for CD10, TdT. LD-PCR for t(8;14)(q24;q32) was negative. AIEOP LNH 97 protocol was started and complete remission (CR) was obtained. Six months after the end of chemotherapy the girl experienced BM and skin relapse. The cell population was more immature with pre-B appearance and low proliferation index. REC 98 HR protocol induced second CR. The girl is now alive and well 8 years after relapse. BM blast karyotype at relapse was 46,XX, t(1;8)(q32;q24). FISH analysis with MYC probe confirmed the rearrangement. Abnormalities of chromosome 1q are common in all forms of B-cell malignancy, mostly as partial duplication of the region: FISH analysis has shown that some of these duplications can be complex. Molecular studies to identify a new non-IG MYC partner that could explain the peculiar clinical history of our case are ongoing.

P07.24 New chromosomal translocations in patients with primary myelodysplastic syndrome

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Cytogenetic analysis is one of the essential methods for determining chromosomal aberrations in myelodysplastic syndrome (MDS). Since more than 70% of patients with MDS have clonal cytogenetic abnormalities, cytogenetic studies play a pivotal role in defining the concept of primary MDS, establishing the diagnosis, evaluating prognosis for survival and transformation to AML and approaching the molecular basis of the disease.

Among structural abnormalities, deletions are the most common changes in primary MDS, while many of the patients exhibit aneuploidy, too. Translocations are rarely seen in MDS and involve certain key genes whose disruptions change the control and the balance of proliferation and differentiation of hematopoietic precursors.

We report a group of 6 patients with primary MDS, in whose karyotypes, novel chromosomal translocations were identified by conventional cytogenetic analysis.

Unstimulated bone marrow samples were used for cytogenetic analysis according to the standard method. Chromosomes were HG-banded (HG-modified banded technique) after direct and 24-hour culture preparation.

The karyotypes were:

1. 46,XY,der(X)t(X;1)(q28;q12)[11]/46,XY[9]
2. 46,XY,der(14)t(1;14)(p11;p11)[12]/46,XY[8]
3. 46,XX,del(20)(q11)[7]/47,XX,t(4;18)(p16;q11),del(20)(q11),+mar[13]
4. 46,XX,-3,t(5;9)(q13;q34),+13[20]
5. 46,XY,del(20)(q11)[5]/46,XY,t(7;20)(q21;q11)[2]/46,XY[13]
6. 46,XX,t(13;22)(q14;q13)[4]/
- 47,XX,del(5)(q13q31),t(13;22)(q14;q13),+21[9]

Determination of the genes involved in the translocation's breakpoints, so as their impact on the clinical picture and prognosis, remain to be

clarified. Cytogenetic results were correlated with other clinical and laboratory parameters and will be presented in the future.

P07.25 A case of angioimmunoblastic T-cell non-Hodgkin lymphoma with a neocentric chromosome inv dup(1)(qter->q21::q21-->q31-->neo-->q31-->qter)

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Neocentromeres are rare epigenetic phenomena in which functional centromeres are formed onto novel chromosomal locations without any alpha-satellite DNA. To date, constitutional human neocentromeres have been reported in at least 90 cases. In cancer, however, the knowledge is much more limited. Acquired neocentromeres have been described in a particular class of lipomatous tumors (atypical lipomas and well-differentiated liposarcomas, ALP-WDLPS), 3 cases of acute myeloid leukemia (AML), 1 case of non-Hodgkin lymphoma (NHL) and 1 case of lung carcinoma. Here, we report a 66-year-old male with angioimmunoblastic T-cell NHL. Cytogenetic analysis of his bone marrow showed multiple aberrations including the presence of a supernumerary chromosome. Using the FISH-technique, the supernumerary chromosome showed to represent an inverted duplication of the segments between 1q21 and 1qter. A neocentromere is formed in band 1q31. To our knowledge, this is the second reported case of NHL (both T-cell) with the presence of a neocentromere. The occurrence of neocentromeres in tumor cells, however, may be underestimated due to technical limitations during the routine diagnostic chromosomal analysis. The prognostic impact is therefore currently unknown.

P07.26 Investigation of aneusomy of chromosome 17 and its influence on prognosis (some clinical and immunohistochemical parameters) amongst some Iranian women with sporadic breast cancer

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Breast cancer is amongst the leading causes of death in women worldwide and the most common cancer amongst Iranian women. Unfortunately, the current clinical and histological criteria can only help 60% of women with breast cancer in diagnosis and long term treatment. Therefore, genetic markers both at single gene level and chromosomes can play an important role in improving the diagnosis and prognosis of breast cancer patients. The aim of this retrospective study was to investigate the role of chromosome 17 copy number assessed by Interphase Fluorescence In Situ Hybridization (FISH) using paraffin embedded breast tumor blocks. The study was carried out on 50 Iranian women, with sporadic primary invasive ductal breast carcinoma. The age range was 31-84 years with an average age of 48.58. The prognostic value of aneusomy 17 in relation to the established clinicopathological parameters, the immunohistochemical markers of ER, PR, P53, age and the duration and status of survival in the patients were investigated. The maximum rate of aneusomy 17 was 83% and the minimum rate was 39% with a mean rate of 57%. The only positive significant correlation was between monosomy 17 and negative status of ER in the patients ($p<0.05$). The relationship between the status of aneusomy 17 and all the studied clinicopathological parameters will be discussed.

P07.27 Chromosomal aberrations in blood cancers

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Cytogenetic studies are important diagnostic and prognostic tool for evaluation of therapeutic procedures among 275 hematological patients, during the period of 2005-2008. in Center for Genetics of Medical faculty - University of Sarajevo. Most of malignant hematological diseases are detectable by chromosomal analyses. Many chromosomal

abnormalities are associated with particular subtypes of leukemia and have prognostic significance. Chromosomal preparations were made by standard techniques and GTG band. We have detected numerous abnormalities among analyzed materials as: t(1:18), t (8:13), t (1:19), t(3:14), t (3:21), t(9:22), inv(16), del (5), inv (3), inv (11) (p11;q23), izo (17), der (11), der (9), tris (8), del (6q), del (13q), that have relevant significance for patients. Cytogenetic studies are important to hematologists to predict good or poor response to treatment in patients with different types of leukemia. The table below shows the number and percentage of abnormal karyotypes among our patients.

| Types of leukemia | Number and % of normal karyotype | Number and % of abnormal karyotype |
|-------------------|----------------------------------|------------------------------------|
| ALL | 34 (12,36%) | 12 (4,36%) |
| AML | 36 (13,09%) | 5 (1,81%) |
| CML | 86 (31,27%) | 19 (6,90%) |
| MDS | 32 (11,63%) | 0 |
| MPS | 17 (6,18%) | 3 (1,09%) |
| Lymphomas | 26 (9,45%) | 5 (1,81%) |
| TOTAL | 231 (84%) | 44 (16%) |

P08 Statistical genetics, includes Mapping, linkage and association methods

P08.01 MMP2 (-1306 C/T) gene polymorphism and susceptibility to abdominal aortic aneurysm or aortoiliac occlusive disease

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Abdominal aortic aneurysm (AAA) is a life-threatening condition affecting 4-9% of population with a risk increasing with age. Aortoiliac occlusive disease (AIOD) is characterized by aortoiliac obstruction and is caused by advanced atherosclerosis. Both AAA and AIOD are considered to have multifactorial etiology and are the most frequent reasons for vascular surgery procedures within abdominal cavity. Destructive remodeling of extracellular matrix related to activity of various matrix metalloproteinases associates both of these pathologies. Matrix metalloproteinase 2 (MMP2) plays a major role in the remodeling of extracellular matrix in arterial vessels and increased MMP2 expression in AAA walls was shown in several studies. Different MMP2 gene polymorphisms were tested in genetic association studies of vascular diseases. The purpose of the present study was to determine if there is association between the functional polymorphism in a promoter region of MMP2 gene (-1306 C/T) and susceptibility to AAA or AIOD in Polish patients.

Based on PCR-RFLP analysis MMP2 genotypes (C/C; C/T; T/T) were determined in three selected groups: 253 patients with AAA and 282 patients with AIOD who underwent surgery; 367 individuals from control group. Genotypes were compared with demographic and clinical data of subjects and analyzed in relation to risk factors. The genotype distribution and allele frequency of MMP2 (-1306 C/T) gene polymorphism were significantly different between patients with AAA and control group. No significant differences were found between patients with AIOD and control group. **Conclusion:** MMP2 (-1306 C/T) gene polymorphism may be an important factor in development of AAA.

P08.02 Absence of association between genotypes at position -262 of ACE and Alzheimer's disease

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ACE is a candidate gene for susceptibility to the sporadic form of Alzheimer's disease (AD). ACE consists of 25 exons at locus 17q23, and encodes angiotensin converting enzyme. The major role of ACE relates to maintenance of circulatory homeostasis. However, it has been demonstrated that ACE can degrade amyloid beta peptide in vitro. This peptide is the constituent of amyloid plaques, the hallmark of Alzheimer's disease. Several variations in the ACE gene have been reported to be associated with incidence and age at onset of AD. Con-

flicting results in this regard have been published with respect to variant SNP rs4291 at position -262 upstream of ACE in its promoter region. In the present research, we aimed to investigate the link between rs4291 and late onset Alzheimer's disease through a case-control study among Iranians. One hundred patients affected with AD with age at onset of more than 65 were selected as cases. The controls were 100 age-matched non-demented individuals. All cases and controls were screened for A/T variations at position -262 by an allele specific PCR protocol. No association between genotypes at this position and Alzheimer's disease status was found.

P08.03 The angiotensin converting enzyme I/D polymorphism and physical performance.

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Bacground: There is an assumption that ACE I/D polymorphism represents one of the possible genetic factors that might be associated with athletic excellence. Recent studies identified that an increased frequency of I allele amongst elite endurance athletes, long distance runners, rowers and mountaineers.

Material and methods: We examined this hypothesis by determining ACE I/D allele frequency by PCR analysis amongst 126 children after motoric tests using lokomometer. ACE genotype and allele frequencies were compared with 252 healthy controls.

Research came into following results: ACE genotype frequency in the whole cohort did not differ from that in sedentary controls ($p<0,56$). We have found positive genotype-dependant association between I/D polymorphism and results of motoric tests in 126 children. There was an excess of the I/I genotype and I allele incidence in group ($n=31$) of endurance types (regarding the speed and frequency: $p\leq0,05$, $p\leq0,01$, respectively).

Conclusion: The frequency of the ACE I/I genotype and I allele was significantly increased among endurance types of children, thus we could conclude that the ACE I/D polymorphism may represent a genetic factor that contributes to the development of elite athletes. The I allele does seem to be associated with enhanced endurance performance, probably via a local muscular effect. Further research might clarify the exact relationship between ACE I/D polymorphism and ACE expression in skeletal muscle and the interaction of this with training, capillary density and substrate utilization. The understanding of the mechanisms of cellular efficiency might have important applications beyond the world of extreme endurance sports.

P08.04 Association of ACTN3 genetic variants with physical performance of Lithuanian athletes

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Homozygosity for the nonsense mutation 577X (c.1729C>T) within ACTN3 results in deficiency of α -actinin-3 but does not result in an abnormal muscular phenotype. Previous research has found an association of the 577R allele with speed/power performance while the 577X allele may confer an advantage during endurance events.

The aim of the study was to investigate genotype and allele distribution of ACTN3 gene R577X polymorphism in elite Lithuanian athlete groups (compiled according to the athlete qualification and their sport orientation (N=149)) and controls (unrelated, untrained individuals (N=250)). Genotyping was performed by RFLP. The aerobic capacity of athletes was measured by using VO_2 max. The ACTN3 RX genotype frequencies in the athletes were: RX- 56.4%, RR-30.9%, XX-12.8% ($\chi^2=4.1$, $p=0.043$), in the controls: RX-50.4%, RR-39.2%, XX-10.4% ($\chi^2=2.46$, $p=0.117$). Genotype frequencies differed significantly between the elite and non-elite athletes ($p=0.04$). XX genotype was more frequent in non-elite (19.9%) than in elite (7%) athletes and in speed/power group (16.7%) compared to endurance (10.4%), mix (14.4%) and control groups (10.4%). Irrespective of the athletes genotype VO_2 max value was highest in the speed/power group. RR and RX genotyped athletes had higher VO_2 max values than XX genotyped athletes. The results indicate that in Lithuanian athletes XX and RX genotypes could be associated with higher speed/power while RR genotype could

be associated with endurance. It can be therefore concluded that the absence of a functional *ACTN3* gene in fast-twitch muscle fibers is most likely compensated by *ACTN2* and other enzymes that take part in anaerobic muscle metabolism.

P08.05 Investigation of complement factor H Y402H polymorphism in Turkish patients with age-related macular degeneration

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Age-related macular degeneration (AMD) is a progressive degenerative disorder known to be the major cause of visual loss in the elderly, especially in well developed countries. AMD has a multi-factorial etiology and is determined by both genetic and environmental factors. Family-based and population based studies showed the association between some gene variants and AMD. CFH Y402H variant seems to be one of the most significant genetic risk factor in AMD cases as previously reported by several studies. There is still some conflict among the results of different studies focused on the relation between CFH variant Y402H and AMD in various ethnic groups.

This study inquires association between CFH Y402H polymorphism and AMD development in a cohort of Turkish AMD patients. A total of 95 AMD patients and 87 age-matched control individuals were genotyped for this allelic variation. Y402H polymorphism was detected with PCR-RFLP method.

Genotype frequencies were highly different between case and control groups compared to the heterozygotes carrying the risk allele C (AMD patients 71.6%, controls 54.03%). This findings suggest that heterozygote individuals with CT genotype (O.R=2.13, CI% 1.11-4.15) have increased risk for AMD compared to homozygotes with TT genotype in Turkish population.

P08.06 Association of apolipoprotein E allele-ε4 with late-onset Alzheimer's disease in Mexican Mestizo population

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Alzheimer Disease (AD) is clinically characterized by global, progressive and irreversible loss of mental faculties. Neuropathologically for neurofibrillary tangles and senile plaques; composed mainly by Amyloid β-peptide (Aβ). The apolipoprotein E allele-ε4 is the major known genetic risk factor for late-onset Alzheimer's disease (LOAD), with a gene-dosage effect increasing the incidence and lowering the age of onset according to heterozygote or homozygote ε4 status. The Apoε4 allele enhances the polymerization and aggregation of Aβ and might affect its clearance, increasing Aβ deposition. APOE allelic frequencies vary between different populations, for example, in a Caucasian (ε2: 8%, ε3: 78%, ε4: 14%), Asian (ε2: 5%, ε3: 86%, ε4: 9%), African-American (ε2: 5%, ε3: 76%, ε4: 19%) and Hispanic (ε2: 3%, ε3: 85%, ε4: 12%).

Materials and Methods: We analyzed the frequency of alleles and genotypes for APOE (ε2/ε3/ε4) and performed a case-control study to evaluate the association between APOE 4 allele with LOAD in Mexican Mestizo population. We studied 77 patients with LOAD and 77 sex and age-matched control subjects. Genotyping of APOE alleles was performed by TaqMan technology. The association risk was estimated using chi-square test and calculation of odd ratios with 95% CI was adjusted by age and sex.

Results: We found different genotype frequencies between cases and controls and observed association for APOE ε4 allele with OR=2.28 (2.17-4.03)

Conclusions: These data suggest a genetic association between ε4 allele with LOAD in Mexican Mestizo population. Our results differ from studies reported in the literature. Acknowledgements: CONACyT (2004-C01-129)

P08.07 Identification of a novel candidate disease locus for anophthalmia/microphthalmia by homozygosity mapping in an Irish traveller family**

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Rare genetic variants associated with disease are, by their nature, difficult to identify by traditional genetic analysis methods. Homozygosity mapping in consanguineous families has been used successfully in the identification of genetic cause in a range of rare conditions. Anophthalmia (absence of an eye) and the related condition microphthalmia (small eye) are relatively rare conditions estimated at 3 and 14 in 100,000 births respectively. The aetiology of anophthalmia/microphthalmia (AM) is not well understood but observed familial clustering suggests a significant genetic component. Several genes have been implicated in AM but, to date, mutations in these genes account for less than 25% of cases. We present homozygosity mapping in a four generation consanguineous Irish family from an endogamous nomadic group presenting with various forms of AM. Previous analysis excluded any causative mutation in known AM genes. Genotyping was undertaken using the high density Illumina 1million SNP platform. Homozygosity mapping identified a 0.9Mb homozygous segment on chromosome 15 shared by all 6 affected individuals but not shared by unaffected relatives. The candidate region contains twelve genes, two of which are strong functional candidates that are implicated in eye development. The candidate region was isolated by NimbleGen's novel sequence capture service and sequence analysis of the region is currently underway. Results of studies into this novel AM candidate region will be presented at the ESHG conference. Identification of a novel AM gene would provide valuable insight into the molecular aetiology underlying the disease phenotype and may enhance our understanding of the disease network.

P08.08 APO E/C1/C4/C2 gene cluster polymorphisms and risk factor for Ischaemic Heart Disease in Spanish population.

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Atherothrombotic diseases are multifactorial diseases caused by genetic and environmental factors. Among the genes that code for apolipoproteins, APO E is located close to APO C1, APO C4 and APO C2 forming the APO E/C1/C4/C2 gene cluster. The APO E plays a major role in lipoprotein metabolism and lipid transport. The three isoforms (apoE2, apoE3, and apoE4) account for a significant proportion of lipid and apolipoprotein variability and have been associated with atherothrombotic disease. As far as the APO C1, APO C4, and APO C2 genes are concerned, they seem to contribute modestly to plasma triglyceride levels. In this work we test 10 SNPs that represent the molecular variability of the APO E/C1/C4/C2 cluster for association with Ischaemic Heart Disease in 101 family trios from Spain with one affected son (N=302 individuals). The results indicate significant disequilibrium in the transmission of the combined C and T alleles (freq=0.36) of SNPs rs405509 (APO E) and rs5167 (APO C4), suggesting that interaction among the elements of the APO E/C1/C4/C2 cluster might also contribute to the development of the disease.

P08.09 Linkage and association analysis for genetic modifiers in a large family with cardiac sodium channel disease.

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To identify loci modulating electrocardiographic (ECG) parameters, we carried out linkage and association analyses at loci harboring 18 candidate genes involved in cardiac electrical activity in two large genealogically-linked kindreds with the sodium channel mutation SCN5A-1795insD (3p21-p25). The mutation leads to the prolongation of several ECG parameters, including P-wave and QRS duration, PQ and QTc interval, ST-elevation as well as slower heart rate. ECGs and DNA were available for 217 individuals (101 carriers). Individuals were genotyped for 1433 tagging SNPs. Linkage and association analyses were performed using SOLAR and PBAT and Kinship, respectively.

As expected, linkage peaks were found for all ECG parameters studied on 3p21-p25 in the region of the mutation (LOD 4.6-19.5). PBAT best association was $p=0.04$ for P wave on 3p21-p25. Kinship found all phenotypes to be highly associated with SNPs on 3p21-p25, with the highest association found for rs11708587 with QTc ($p=1.2e-23$).

Based on the present study, all of the analyses programs tested picked up the major known effect of the mutation although PBAT was outperformed by Kinship, picking up this major effect in all ECG parameters instead of just P-wave. After correcting for our main effect, different loci were found both by linkage and association. Both Kinship and SOLAR perform well in the present large kinship, but due to time consuming IBD calculations, association using Kinship can be regarded as a faster alternative. The lesser performance by PBAT is most likely related to the large size of our kindred.

P08.10 rs4307059 and rs4141463 study in an Italian cohort with Autism spectrum disorder.

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The genetic component of Autism spectrum disorders (ASD) is complex including known chromosomal anomalies, rare genetic variants and copy number variants. More recently, a set of common polymorphisms on 5p14.1 have been associated with ASD in two independent studies (Wang et al, Nature 2009 and Ma et al, Annals Human Genetics 2009) and another SNP on the gene MACROD2 showed association at the genome-wide level (IMFAR, International Meeting For Autism Research 2009).

Thanks to Fondazione SmithKline (FSK), a large multicentric study has been funded with the aim of collecting biological material (DNA, RNA, plasma and lymphoblastoid cell lines) from extremely well-characterized patients, parents and relatives and studying ASD pathology. Till now we have collected 533 individuals for 162 familial units, and it is planned to recruit a total of 350 families within year 2010.

At the moment we have genotyped 486 individuals (144 families) for the two SNPs (rs 4307059, located at 5p14.1 and rs4141463 located in the MACROD2 gene) with allelic discrimination TaqMan Assays. We performed TDT test (UNPHASED software) and we did not find any significant association for any of the two SNPs.

Increasing the number of individuals will allow us to test more complex models of transmission (such as parent-of-origin effect, preferential transmission to males or females, gene-gene interactions) and investigate the correlation of affection status with the numerous available quantitative phenotypes.

P08.11 Study of Autosomal Dominant non syndromic loci in Iranian population with Hearing loss

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Hearing loss is common at all ages and can be due to environmental factors, genetic defects, or a combination of the two. The genetic basis of hearing loss is highly complex. About 70% of all cases of the inherited forms of hearing loss estimated to be non-syndromic, which are most

often sensorineural. Non-syndromal sensorineural deafness is a highly heterogeneous condition, which can be divided into DFNA (autosomal dominant deafness, ~15-20%), DFNB (autosomal recessive deafness, ~80%), DFN (X-linked deafness, ~1%), and mitochondrial deafness (at least 1%). In this study, we selected 20 Iranian families with autosomal dominant pattern of inheritance. All of the patients belonged to large families who had more than 5 affected members in three generations. Using linkage analysis method with flanking and intragenic STR markers, we studied the following loci: DFNA2, DFNA6, DFNA20/26, DFNA9, DFNA8/12, and DFNA3. These genes have a high prevalence especially in neighboring countries of Iran. In fact, they are the most prevalent among DFNA loci. In our previous studies, we could find large deaf families which were linked to DFNA3, DFNA36 and DFNA5 with novel mutations. Continuing this project, So far three of our families have been linked to DFNA2 and DFNA6. Mutation in these genes (KCNQ4, GJB3 and WFS1) has been shown in many populations. The mutation screening for these two families is underway.

P08.12 Investigation on the prevalence of 8 autosomal recessive non syndromic loci in Iranian population with hearing loss

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Hearing loss is the most frequent neurosensory disorder which is highly heterogeneous, and can also occur due to environmental causes. Around 50% of the HL cases in developed countries have genetic origin. However not much investigation has been done in developing countries to show the prevalence of different genes.

Mutations in *GJB2* gene are the major cause of recessive non-syndromic hearing loss in the Iranian population. Recent studies indicated that the mutation in *SLC26A4* gene located in DFNB4, as the most prevalent syndromic form of hearing loss, and mutation in *TECTA* gene, as the second most-prevalent genes involved in non-syndromic in Iran.

In our previous study we demonstrated several mutations for the genes located in the following loci DFNB2, DFNB3, DFNB7/11, DFNB9, DFNB16, DFNB24, DFNB53 and DFNB59 and we could not find any linkage to other previously reported genes. In this study we would like to determine the prevalence of these eight loci in our population. Total of 91 Iranian families were selected with the following criteria: 1) History of two or more affected individuals in the family, 2) Families with NSARHL, who were negative for the DFNB1, DFNB4 and DFNB21 loci. Homozygosity mapping with flanking and intragenic STR markers used to investigate these 8 loci. So far total of 75 families have been investigated of which 5 have been linked to DFNB3, 3 to DFNB24 and one other to DFNB7, 11. The linkage study for remaining families and mutation screening for *MYO15A*, *RDX* and *TMC1* genes are underway.

P08.13 Molecular genetic study of genes biotransformation of xenobiotics

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In this work we examined the polymorphic variants of genes of enzymes of phase II biotransformation of xenobiotics belonging to the family of glutathione S-transferases: *GSTM1*, *GSTP1* gene and the putative tumor suppressor gene *p53*.

The material was analyzed in DNA samples from 594 people. All the studied sample was divided into 2 groups: residents of areas with adverse environmental conditions (189 people) and the comparison group - 405 people.

Analysis of the frequency distribution of genotypes and alleles of polymorphic loci Ile105Val (A313G) and *GSTP1* gene polymorphic variants of *p53* gene (Arg72Pro, dup 16 bp) did not reveal significant differences between the groups ($p > 0.05$).

Analysis of the frequency distribution of genotypes of *GSTM1* gene deletion polymorphism showed a tendency to increase the genotype “+” ($p = 0.0598$, OR = 0.670) and significant increase in the combination of genotypes + / Ile / Val ($p = 0.004$; OR = 2.205) in the group with low ecological load.

At typing in polymorphic variants of p53 gene (Mspl-polymorphism) has been shown that the frequency of genotype ww ($p = 0.017$, OR = 0.634) and allele w ($p = 0.037$, OR = 0.707) higher in the comparison group, and genotype wm ($p = 0.015$, OR = 1.601) and allele m ($p = 0.037$, OR = 1.416) in the group of persons living in conditions of high environmental load. This may indicate that individuals living in areas with high environmental stress, more prone to the emergence of diseases such as cancer.

P08.14 Genome-wide association of breast cancer: composite likelihood with imputed genotypes

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We describe a composite likelihood-based analysis of a genome-wide breast cancer case-control sample from the Cancer Genetic Markers of Susceptibility (CGEMS) project. We determine 14,380 genome regions of fixed size on a linkage disequilibrium (LD) map which delimit comparable levels of LD. Although the numbers of SNPs are highly variable each region contains an average of ~35 SNPs and an average of ~69 after imputation of missing genotypes. Composite likelihood association mapping yields a single P -value for each region, established by permutation testing, along with a maximum likelihood disease location S , standard error and information weight. For single SNP analysis the nominal P -value for the most significant SNP (msSNP) requires substantial correction given the number of SNPs in the region. Therefore, imputing genotypes may not always be advantageous for msSNP testing, in contrast to composite likelihood. For the region containing FGFR2 (a known breast cancer gene) power is greatest under composite likelihood with imputed genotypes (χ^2 increases from 20.6 to 22.7), and the single SNP-based χ^2 is 19.9 after correction. Imputation of additional genotypes in this region reduces the size of the 95% confidence interval for location of the disease gene by ~40%. Amongst the highest ranked regions, SNPs in the NTSR1 gene would be worthy of examination in additional samples. Meta-analysis, that combines weighted evidence from composite likelihood across corresponding regions in different samples and refines putative disease locations, is facilitated through a test for homogeneity. This test is more powerful than methods that combine unweighted chi-squares through probability.

P08.15 Molecular genetic study of genes of cardiovascular system in athletes

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Introduction: Molecular genetics of sport - a relatively young discipline whose task is to search for polymorphisms in genes that lead to individual differences in the development and manifestation of various physical qualities of human. Were typing these genes governing the operation of the cardiovascular system: renin (ren Mbol), which cleaves angiotensinogen and angiotensin form I; chymase (CMA1/B), involved in the conversion of angiotensin I in the enzyme active vasoconstrictor angiotensin II; vascular receptor-widening factor - bradykinin (BDKRB).

Materials And Methods: We studied samples DNA of 145 athletes with high achievement (candidates for master of sports and the master of sports) aged 17-30 years and specialising in different sports. Control group consisted of 144 healthy people who are not involved in sports. The analysis genetic polymorphism is realized by polymerase chain reaction (PCR) and RFLP- analysis.

Results: Analysis of the frequencies of genotypes and alleles of genes bradykinin receptor (BDKRB) and chymase (CMA1/B) revealed no significant differences between the groups studied. Analysis of the gene renin (ren Mbol) in athletes compared with the control group showed a significant increase in the frequency of genotype ren +/- (76.2% against 54.6% in the control group; $P = 0.01$).

Thus, the renin gene (ren Mbol) may be associated with the level of physical performance, but also for identifying favorable allelic variants of genes, providing a more effective implementation of physical work, necessary further study of genetic markers.

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P08.16 Investigating ANKH and ENPP1 in Slovakian families with chondrocalcinosis

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Familial articular chondrocalcinosis was first reported in 1963. It is characterized by multiple calcifications of hyaline and fibrous cartilage in the joints and intervertebral disks. Mutations in ANKH have been identified in several pedigrees as a monogenic cause for this disorder. ANKH is a key protein in pyrophosphate metabolism since it is the responsible for pyrophosphate transport across the cell membrane. The objective of this work was to screen ANKH and ENPP1, two key genes in pyrophosphate metabolism, in Slovakian kindreds with familial chondrocalcinosis.

DNA samples from 25 individuals (10 affected, 15 unaffected) from 8 families were obtained. The promoter, coding regions and intron-exon boundaries of ANKH and ENPP1 were sequenced.

Twelve DNA sequence variants, six in each gene, were identified. All the variants had been previously identified. None segregated with the disease. Due to the high rate of homozygous parents (affected and unaffected) the genetic informativeness of these families was scarce for association studies. Our results suggest that neither ANKH nor ENPP1 mutations are the cause of chondrocalcinosis in these families, indicating that possibly other major genes are involved in the aetiopathogenesis of this condition. A possible role of variant K121Q of ENPP1 needs to be further investigated.

P08.17 Evidence of association between GDF5 polymorphisms and congenital dislocation of the hip in a Caucasian population

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Congenital dislocation of the hip (CDH) is a multifactorial disease which involves genetic factors that are still unidentified. Recently, a functional polymorphism (rs143383) of the 5'UTR region of GDF5 (Growth/Differentiation Factor 5), previously reported to be associated with osteoarthritis, has been associated with CDH in a Chinese population. The aim of our study was to determine whether GDF5, known to be involved in bone and joint morphogenesis, is also associated with CDH in Caucasians. We genotyped three tagSNPs (rs224334, rs143384, rs143383) in 239 cases and 239 controls from western Brittany (France) where CDH is frequent, and tested the association using both single-locus and haplotype-based approaches. The most significant association was observed with rs143384. The T allele of this SNP was overrepresented in cases (65.9% vs. 55.9%, $p=0.002$). Under a recessive model, carriers of the TT genotype had a 1.71-fold higher risk of developing CDH than carriers of the other genotypes (OR TT vs. CT+CC=1.71, 95% CI: [1.18-2.48], $p=0.005$). At a nominal level, the association was also significant with rs143383 (OR TT vs. CT+CC=1.52, 95% CI: [1.05-2.19], $p=0.026$). The haplotype carrying the susceptibility alleles of these SNPs was also more frequent in cases (65.9% vs. 55.9%, OR=1.53, 95% CI: [1.18-1.98], $p=0.002$). This study reports, for the first time, the association between GDF5 polymorphisms and CDH in Caucasians, and points out another polymorphism of interest that requires further investigation. Reduction in GDF5 expression might lead to developmental deficiency of ligaments and capsule in hip joint, and therefore contribute to CDH pathogenesis.

P08.18 The spectrum of the most common CFTR gene mutations at Kaunas Medical University Hospital

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Cystic fibrosis is one of the most common autosomic recessive disease with a prevalence of 1:1600-1:4000 in Europe. It presents mainly with pulmonary or/and gastrointestinal symptoms and accounts for a small portion of male infertility. 95 patients were referred for genetic

counseling with suspicion of cystic fibrosis (CF) diagnosis between 2004-2009. The spectrum of most common mutations was tested with the help of INO-LIPA CFTR 19 mutation detection kit (Innogenetics, Belgium).

Results: The CFTR gene mutations were detected in 23.16% from all analyzed cases. The most common mutation, as expected, was F508del with a incidence of 81.82% out of all detected mutations. 3 patients were diagnosed to have CFTRdel2,3 (21kb) mutation, that accounts for 13.64% out of all detected. R553X mutation showed the incidence of 4.54% (1 patient). As far as F508del is concerned, 4 patients were diagnosed to be homozygotes (F508del/F508del) and 10 heterozygotes (including one patient with detected 2 mutations : F508del and CFTRdel2,3). One patient was heterozygote for another two mutations: R553X and CFTRdel2,3. In conclusion the spectrum of CFTR gene mutations was similar to that reported for European population, with especially high incidence of F508del.

P08.19 Role of DRD4 Polymorphism in Attention-Deficit Hyperactive Disorders

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Attention-Deficit Hyperactivity Disorder (ADHD) is characterized by hyperactivity and difficulty in sustaining attention and impulsivity. Defiant and various anxiety disorders, depression, learning disorders, mood disorders, alcohol and drug abuse may coexist with ADHD. Previously, several SNPs in different genes have been effect on characteristic of ADHD. In this study, DRD4 polymorphism investigated in the persons (n=90) whom have responsible for two and more traffic accidents. Genotyping and allelic distribution compared with control group (n=100) who has no traffic accident history as driver. Adult ADHD Self-Report Scale (ASRS) test was performed to determine attention and/or hyperactivity disorders. DRD4 intronic polymorphism (rs752306) determined in both of the groups. No significant difference in the allelic distribution has been found between the groups.

P08.20 A family-based association test to detect gene-gene interactions in the presence of linkage

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For many complex diseases, quantitative traits contain more information than dichotomous traits. One of the approaches used to analyse these traits in family-based association studies is the Quantitative Transmission Disequilibrium Test (QTDT). The QTDT is a regression-based approach that models simultaneously linkage and association. It splits up the association effect in a between- and a within-family genetic component to adjust and test for population stratification and includes a variance components method to model linkage.

We extend this approach to detect gene-gene interactions between two unlinked QTLs by adjusting the definition of the between- and within-family component and the variance components included in the model. To capture the influence of population stratification, we derive the bias of the estimated interaction effect and discuss the influence on type I error rates. We simulate data to investigate the influence of the epistasis model, LD patterns between the markers and the QTLs, family structures and allele frequencies on the power and type I error rates of the approach. Results show that for some of the investigated settings, power gains are obtained in comparison with other techniques (e.g. FBAT-LC/FBAT-MM). We conclude that our approach shows promising results for studies where too few markers are available to correct for population stratification using standard methods (e.g. EIGENSTRAT). The proposed method is applied to real-life data on hypertension.

P08.21 Choosing GWAS subjects from risk populations

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Power, i.e., sample size is a crucial issue in genome-wide association studies (GWAS) on disorders generated by a multitude of weak genetic effect sizes. Here, we examine the influence of sampling cases and/or controls from populations that are subject to an external risk factor

(such as smoking, nutritional or metabolic factors). We use an additive threshold model and derive the necessary sample sizes as a function of the external risk factor's strength and of the sampling scheme. If both cases and controls are sampled from the risk population, a loss of power must be expected. The loss of power (i.e., increase of necessary sample size) is even larger if only the cases are sampled from the risk population, while the inverse scheme (non-risk cases and risk controls) provides a gain of power since non-risk cases are enriched for disease-favouring alleles while risk controls are enriched for protective alleles. For small effect sizes we derive simple approximations in analytically closed form. The principle of our analysis has some analogy to the so-called Carter effect, i.e. the phenomenon that the probability in a child of a multifactorial disorder with sex dimorphism for liability depends on the sex of the affected parent. In summary, we propose to optimize GWAS sampling from risk populations in order to minimize the necessary sample sizes.

P08.22 Segregation exclusion analysis (SEGEX) efficiently identifies genomic regions harboring germ line mutations in familial disorders

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Recent advances in high-throughput microarray genotyping have facilitated research in many fields of genetics, including disease gene identification in familial disorders. We developed a simple and robust algorithm for non-parametric linkage analysis based on SNP array data. The algorithm is based on identification and exclusion of all genomic regions that are not shared between affected members of the family, while remaining non-excluded regions are called. Genotype data of more than 900,000 SNPs used in the analysis were obtained from Affymetrix Genome-wide SNP 6.0 arrays. The method uses only affected members of the family and does not take into account mode of inheritance or level of penetrance. We have validated the algorithm by analyzing three families with known disease-causing mutations: one family with hereditary parkinsonism caused by mutation in *LRRK2* gene, two - with hereditary thrombocythemia caused by mutations in thrombopoietin (*THPO*) gene. In family with parkinsonism and one of the families with thrombocythemia the algorithm showed single genomic region in linkage, which contained *LRRK2* and *THPO* genes, respectively. In the other family with thrombocythemia, there were 3 regions identified due to smaller family size, *THPO* located in one of them. In general we observed negative exponential correlation between the total size of called genomic regions and the number of meioses separating all family members analyzed. Overall, SEGEX performs well with high density SNP genotype data, precisely defining regions containing disease-causing mutations and is a useful tool for disease gene mapping.

P08.23 Familial amyloid polyneuropathy (FAP) ATTRV30M: a good model for the study of genetic modifiers for age-at-onset (AO)

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Familial amyloid polyneuropathy (FAP) ATTRV30M is an autosomal dominant systemic amyloidosis, due to a point mutation in the transthyretin (TTR) gene (chr18q12.1). It was first described by Andrade in Northern Portugal, in 1952, as a disease beginning mainly between 25 and 35y of age. It was later shown to present a much wider variability in AO, among different clusters (Portugal: 34.7, Sweden: 56.7, Majorca: 45.7), but also within the same focus. In CHP (Porto), 2331 patients belonging to 550 different families were diagnosed from 1939 to 2008. We have previously shown that, in our series, AO varied from 20-80yrs (mean: 37.1 in women, 32.4 in men). We also reported that 209 probands (40%) had no affected parent at the time of diagnosis, and that these had a much later AO (46.5y) than the ones belonging

to families with one affected parent (32.3y). We now studied sex differences between probands with or without an affected parent: interestingly, while female probands with an affected parent had a later onset (35.8y) than males (30.3), this difference was not significant for probands with no affected parent (46.9 and 46.2, respectively). The most intriguing feature was the fact that this protection very often fades away and next generation manifests with classical AO. Moreover, this protection seems to be lost permanently, since in our 70 years' registry, we never found classical onset parents giving origin to late-onset offspring. A cis-acting effect, within or close to the TTR locus, may be responsible for some variation between generations.

P08.24 Assosiation study between IL1RA gene polymorphism and Febrile Convulsion in Iranian children

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Background and aim: Fever and seizure is the one of the most reason for children registration in hospitals. Some children as their genetic structure have less resistance to fever, as recent studeis express positive corelation between family history for febrile convolution (type and age of onset in children) and disease. So, this study was performed to survey about assosiation between Inter Leukine 1 Receptor Antagonism gene, IL1RA gene polymorphism and predisposition to disease. Method: In this case - control study 100 febrile convolution (FC) patients that refered to pediatric department and emergency ward of Hajar hospital and also 130 healthy children were selected. The age average of the patients group was $3/4 \pm 1/4$ years and for the control group was $3/4 \pm 1/2$ years. 44 cases of the patients group had a positive history for FC. After sampling, DNA extraction and PCR reaction for IL1RA gene was performed. Finally by the comparision of segments size results were analysed.

Results: The genotypes frequency of the IL1RA gene allele1 and 2 in the patients group was 56% and 10%, and for control group was 55/4 % and 6/9% respectively.

Conclusion: Considering to $P = 0/93$ for allele 1 and $P= 0/401$ for allele 2, there was no significance difference between two groups. Based on the chi square test, there was no correlation between IL1RA gene polymorphism and predisposition to F.C. disease.

P08.25** Multiple Marker Methods for Analysis of Genome Wide Association Studies

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Genome Wide Association Studies have had success at identifying the genetic causes of complex trait variation, but generally explain only a small fraction of the genetic variance indicating a number of undetected variants. We investigated the power of different regression models to identify associations across the range of minor allele frequencies (MAF) for the causative variant. We explored the performance of linear regression models on simulated traits using single or multiple (3, 5 or 7) adjacent genotyped markers, haplotypes consisting of 3, 5 or 7 adjacent markers and single imputed markers. We analysed data from a single real chromosome of 453 unrelated individuals genotyped with the Illumina's HumanHap300 Genotyping BeadChip comprising 317,503 SNPs. We simulated 1,000 traits by omitting 100 genotyped SNPs in each of 10 MAF classes (<0.05 to >0.45). In each dataset one of the SNPs was treated as causative and set to explain 0.3 of the trait variance. The genotyped regressions analysed 16,024 markers, while 163,157 imputed markers (MACH1.0; CEU HapMap2 as reference population) were analysed. We compared the ability of the methods to identify the simulated causative variant on the basis of the significance of the association. The three-marker method using genotyped data performed well at MAF >0.1 , but below that the haplotype methods supersede. The significance of analyses using imputed SNPs was greater than analyses using genotyped SNPs for MAF <0.2 . However, the advantage was slight and has to be weighed against the 10-fold increase in the number of tests performed using the imputed data.

P08.26 Biotransformation gene polymorphisms and the risk of glucocorticoid-induced osteoporosis

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Glucocorticoid-induced osteoporosis (GCOP) is the most common cause of secondary osteoporosis in long-term use of corticosteroids, which leads to significant morbidity and mortality. Glucocorticoids are widely prescribed drugs, especially in the treatment of allergies, thus, the study of risk factors for GCOP in patients with bronchial asthma receiving long-term systemic corticosteroid therapy is an important problem of prevention medicine.

The aim of this work was to determine the genetic component in the GCOP etiology by the analysis of 8 candidate gene polymorphisms together with quantitative indicators of Bone Mineral Density (BMD) and Z-criterion in 137 Russian patients with asthma receiving long-term systemic corticosteroid therapy. The analysis of biotransformation gene polymorphism was performed using the low-density "Pharma-gen-biochip".

At comparison of BMD indexes of proximal femur for the Z-criterion revealed a statistically significant association of gene polymorphism *MTHFR* 677C>T (non-parametric ANOVA Kruskal-Wallis $p = 0.0013$). In addition, for the insertion-deletion polymorphism of *GSTM1* also was found statistically significant differences by Mann-Whitney U-test ($p = 0.016$). Interestingly, that the *GSTM1* and *MTHFR* genes are localized on 1p chromosome (1p13.3 and 1p36.3 respectively), so we can suggest that the chromosome region may be involved in the GCOP susceptibility in Russia.

The determination of the *GSTM1* and *MTHFR* alleles seems to be important to identify group of patients with high risk for osteoporosis among individuals receiving long-term systemic corticosteroid therapy for the timely prevention of disease.

The work was supported by the Russian Foundation for Basic Research (project no. 08-04-12225).

P08.27 INTERSNP: Genome-wide Interaction Analysis Guided by A Priori Information

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Genome-wide association studies (GWAS) have lead to the identification of hundreds of genomic regions associated with complex diseases. Nevertheless, a large fraction of their heritability remains unexplained. Interaction between genetic variants is one of several putative explanations for the "case of missing heritability" and, therefore, a compelling next analysis step. However, genome-wide interaction analysis (GWIA) of all pairs of SNPs from a standard marker panel is computationally unfeasible without massive parallelization. Furthermore, GWIA of all SNP triples is utopian. In order to overcome these computational constraints, we present a GWIA approach that selects combinations of SNPs for interaction analysis based on a priori information. Sources of information are statistical evidence (single marker association at a moderate level), genetic relevance (genomic location) and biologic relevance (SNP function class and pathway information). We introduce the software package INTERSNP that implements a logistic regression framework as well as log-linear models for joint analysis of multiple SNPs. Automatic handling of SNP annotation and pathways from the KEGG database is provided. In addition, Monte-Carlo simulations to judge genome-wide significance are implemented. We introduce various meaningful GWIA strategies that can be conducted using INTERSNP.

Typical examples are, for instance, the analysis of all pairs of non-synonymous SNPs, or, the analysis of all combinations of three SNPs that lie in a common pathway and that are among the top 50,000 single-marker results. We demonstrate the feasibility of these and other GWIA strategies by application to a GWAS data set and discuss promising results.

P08.28 Haplotypes analysis of VDR gene and spinal muscular atrophy disease

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Aim: To investigate the relationship between VDR gene haplotypes and SMA disease.

Materials and methods: Blood samples were collected from SMA patients (n=70), and control subjects without heredo-collateral or personal antecedents (n=54). The homozygous deletion of SMN1 gene was detected in all the patients, meanwhile at least one copy was identified in the control lot. The haplotypes of VDR gene polymorphisms (*FokI*, *BsmI*, *Apal*, *TaqI*) were inferred after PCR-RFLP genotyping, using SHEsis platform.

Results: A significant relationship was detected in case of fbaT haplotype which seems to confer risk for SMA type I disease only (OR=2.801, 95%CI: 1.139-6.887, p=0.02). Taking into account that only two SMN2 copies were found in SMA type I patients, we can speculate either a relationship between VDR haplotypes and SMN copies number. The results suggested also the importance of performing haplotypes rather individual marker analysis, as the single locus study detected a significant relationship only for *BsmI* polymorphism.

For the patient's lot, we didn't identify a strong linkage disequilibrium relationship regarding *BsmI*, *Apal*, *TaqI* polymorphisms, as it is usually reported. Random sampling or recombination events that could affect other genomic regions than 5q11.2-13.3, might be possible explanations for our results. Regarding the control lot, the most prevalent three locus haplotypes are bAT (31.4%), BAt (28.1%) and bAT (18.2%), as it was previously reported in Caucasian population.

Conclusions: The analysis of VDR gene haplotypes should be taken into consideration in the studies regarding possible modifier factors of SMA disease.

Study supported by grant CNCSIS-IDEI 2152/2008.

P08.29 Comprehensive approach to investigate the genetic basis of Hereditary Hearing Loss in Iranian population

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Genetic testing for deafness in Iran is well established. The population is extremely heterogeneous, which means that to determine the genetic basis of hereditary hearing loss in the Iranian population, ethnic-specific data are required. Over that last several years, We have generated and published much of these data by screening over 2000 families segregating autosomal recessive non-syndromic deafness (ARNHL). All patients were screened for mutations in *GJB2* and *GJB6* in the Deafness Neurosensory Type 1 (DFNB1) locus, and if no mutations were identified, haplotype were reconstructed by typing three short tandem repeat polymorphisms flanking 24 known ARNHL loci. In a subset of families, genome-wide linkage analysis was completed. The most prevalent mutation in this gene was 35delG, although we identified 34 different mutations, seven of which are common. We have also identified a novel *GJB2* mutation in an endogenous population with autosomal dominant non-syndromic hearing loss (ADNSHL) in a village in North of Iran. In approximately 50% of these families, we have been able to establish genetic causes for deafness. About 15% have mutations in *GJB2* followed by mutations in *SLC26A4* and *TEC-TA*. We have also found mutations in *PJVK*, *TMC1* and *USH1C*, *OTOF*, *MYO11A*, *MYO15A*, *COLLA2*, *RDX* and *VLGR1*. Finally, we have described a new syndrome, a contiguous gene deletion syndrome that involves both Deafness and Infertility Syndrome (DIS) in males. These data from the Iranian population attest to its diversity and contribute to the current body of knowledge regarding the deafness of genetics.

P08.30 Genetic background of Severe course of IBD

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Inflammatory bowel disease (IBD) is characterized by chronic relapsing inflammation in gastrointestinal tract. IBD is divided into two subtypes Crohn disease (CD) and ulcerative colitis (UC). If it is not possible to differentiate these IBDs the patients are diagnosed as indeterminate colitis (IC). In our 160 severe IBD patients with average age of diagnosis 26 years, the youngest patient was diagnosed when was 3 years old and the oldest one was diagnosed at the age of 69. In this group we investigated frequency of alleles in NOD2/CARD15 gene and 15-PGHD gene. The 15-PGHD gene codes dehydrogenase which is a prostaglandin-degrading enzyme and acts as an antagonist to enzyme called cyclooxygenase 2. We also studied frequency of haplotype in q31 region on 5th chromosome. We estimated frequency of alleles SLC22A4 1672T and SLC22A5 T207C. In studied group we observed increased frequency of INV4+39C>T 15-PGHD homozygotes in group of patient under 18 years old with UC (12%) in comparison to adult patients where the INV4+39C>T 15-PGHD homozygotes were not been observed. The frequency of A at position 168 in PGHD gene were higher in patient under 18 years old (49%) than in adult patients (34%). In NOD2 gene we observed statistically significant differences of frequency of P268S, R702W and 2030insC in group of patient with severe course of IBD in comparison to unselected IBD patients and control group. The study was supported by the Polish Ministry of Science and Higher Education projects no. 2P05A06929.

P08.31 Software for multipoint parametric linkage analysis of quantitative traits in large pedigrees

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Parametric linkage analysis is one of the most powerful tools for mapping of traits with known model of inheritance. Several well-known programs may be used for this analysis. However these programs do not work with the large extended pedigrees from genetically isolated human populations and great part of them cannot analyze quantitative traits. Large pedigrees with thousands members are available now for genetic analysis. Utilizing such pedigrees in linkage analysis is computationally challenging.

We developed software for multipoint parametric linkage analysis of quantitative traits using information about SNP genotypes. Mixed model of major gene and polygene inheritance is implemented in this software. Implementation of several algorithms to avoid computational underflow and decrease running time permits application of our software to the analysis of very large pedigrees collected in human genetically isolated populations. The software assigned for the multipoint parametric linkage analysis based on both known (estimated prior to linkage) model parameters and the ones estimated during the analysis. We tested our software by performing linkage analysis of adult height in large pedigree from Dutch isolated population. Three significant and four suggestive loci were identified with the help of our programs, whereas VC based linkage analysis, which requires the pedigree fragmentation, demonstrated only three suggestive peaks.

The software package MQScore_SNP is available at <http://mga.biobnet.nsc.ru/soft/index.html>.

P08.32 Multipoint linkage disequilibrium mapping with incorporation of covariates in general pedigrees

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Complex diseases are often involved a number of genetic and environmental factors. Incorporating the factors relevant to a complex disease as covariates into the disequilibrium mapping can therefore enhance the efficiency of a disease locus estimate as well as help dissect the etiology of a complex disease. Previously, we developed two approaches to incorporate covariates into linkage disequilibrium mapping in the case-parent design. The approaches, including parametric and non-parametric modeling, are robust in that no assumption about

the underlying genetic model is required, other than the assumption that there is no more than one disease gene in the chromosomal region. In addition to the estimate of a disease locus, the magnitudes of the associations between the genetic effect at the disease locus and covariates can also be assessed.

In practice, data are often available for larger pedigrees with multiple nuclear families or general pedigrees, it would be desirable to have the association mapping approaches that can use all potentially informative data. In the present study, we will extend these approaches to general pedigrees to make full use of the data available, so as to improve the efficiency of estimate for a disease locus. By making full use of pedigree data, one can also estimate the relative risks among different relatives, which is informative for uncovering the underlying genetic model of a disease.

P08.33 Study of polymorphic variants of genes LPL (Hind III, S447X) and CETP (Tag1B) in individuals with different levels of total cholesterol in the blood

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Lipoprotein lipase gene LPL (8p22) and the gene ester transfer protein cholesterol CETP (16q21) is one of the major genes involved in the regulation of lipid metabolism. Studied polymorphic variants of the HindIII, S447X LPL gene and Tag1B gene CETP, as well as an analysis of associations studied polymorphic variants of the level of total cholesterol (TC) of human blood plasma.

Material for the study included DNA samples from 380 healthy individuals. Determining the level of cholesterol carried by standard enzymatic methods. Analysis of DNA polymorphic loci of LPL and CETP performed by PCR-RFLP.

Results: In the group of persons with high cholesterol showed significantly significant increase in the genotype LPL (Hind III) H+/H+ in blood (39.19% versus 25.61% in the group of individuals with indicators of cholesterol in normal, P=0,031), and also showed a trend to increased CETP genotype B1/B1 (34% vs. 25%, respectively, P=0.070). Revealed a statistically significant increase of genotypes in the study of polymorphic variations in a group of persons with high levels of cholesterol (a variant of H+H+/SS/B1B1, P=0.04).

Conclusions: We found that polymorphic markers Tag 1B in the gene ester transfer protein CETP cholesterol and Hind III in the lipoprotein lipase gene LPL associated with the level of cholesterol in the blood, and the genotype of CETP B1/B1 and LPL genotype H+/H+ are markers of high levels of cholesterol in human blood.

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P08.34 A variant in EDNRA is associated with migraine without aura in a group of Portuguese patients

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Migraine is a common disabling primary headache, leading to a diminished quality of life in migraineurs and their relatives. Anomalies of vascular function, with dilatation of cerebral blood vessels and release of vasoactive neuropeptides have been implied in its pathophysiology. Endothelin type A receptor (EDNRA) mediates the biological effects of endothelin-1 (ET-1), leading to vasoconstriction. Our aim was to assess the involvement EDNRA in susceptibility to migraine in a sample of Portuguese migraineurs, by a case-control study.

Three tagging SNPs (rs702757, rs5333 and rs5335) were analyzed in 188 cases - 111 without (MO) and 77 with aura (MA) - and 287 controls. A multivariable logistic regression included the three SNPs, adjusted for gender. Allelic and haplotypic frequencies were compared between cases and controls.

We found a borderline increased risk for the rs702757 T-allele (OR=1.44, 95% CI:1.05-1.99) and for the TT genotype (OR=2.34, 95% CI: 1.12-4.90) for MO. A trend towards an increased risk for MA regarding the C-allele of rs5333 was also found. The T-C-G haplotype was found to be significantly overrepresented in the MO subgroup.

Our results reinforce EDNRA as a susceptibility factor for MO, although we cannot exclude the involvement of this gene in MA susceptibility in our population. Dissecting migraine genetic susceptibility will be crucial to develop better therapeutic strategies, as the risk variants implied and their effects may vary in different populations.

P08.35 Associating mitochondrial DNA variation with complex traits

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Saxena and colleagues produced a set of SNP's for testing association between common mitochondrial DNA [mtDNA] variation and disease; these SNP's have continued to be used publications. Despite concerns however that the SNP's are not sufficiently accurate or comprehensive. The SNP's were derived from publicly available human mtDNA sequences and intended to represent diversity in European populations. However, 101 (~8%) non-European sequences were included and ~16% were from the Finnish population, where rare mtDNA clades are overrepresented. Furthermore, ~11% of the mtDNA sequences were from patients with type 2 diabetes, Alzheimer and Parkinson disease, although many studies have hunted for association of these diseases with mtDNA SNP's.

The SNP's derived from the above data reliably detect some common haplogroup-defining polymorphisms and thus some haplogroups but not others. The SNP's included many homoplasic, i.e. changes that have occurred more than once on the phylogeny. The majority of established mtDNA haplotypes are defined by a combination of SNP's. Some haplogroups are tagged by a single homoplasy using the Saxena SNP's; by definition a single homoplasy cannot reliably "tag" a single mtDNA haplotype. Of the SNP's identified by Saxena et al. 53% were homoplasic associated with two or more haplogroups. In short, mathematically sound methodology was applied but the data was flawed and failing to apply knowledge about the evolution of mtDNA resulted in the production of SNP's not well suited to the purpose. Careful selection of SNP's using knowledge of evolution and biochemical effect will be needed to progress.

P08.36 Skew in the human caveolin 1 gene upstream purine complex homozygote haplotype compartment in multiple sclerosis

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Caveolin 1 (CAV1) is a component of the myelin sheath and the expression of the gene encoding this protein is increased during myelination in Schwann cells and oligodendrocytes. We sought to investigate the homozygote haplotype compartment in a recently identified polymorphic purine complex at the upstream region of the human CAV1 gene in multiple sclerosis (MS). In a case/control study design, the region was characterized in 126 cases of MS diagnosed based on the Revised McDonald diagnostic criteria, and 460 controls. We report a skew in the homozygote haplotype compartment in the cases versus controls both in a qualitative and quantitative respect. Excess homozygosity for haplotypes was observed in the MS cases (corrected $p<0.012$, OR=2.54, CI 1.14-5.64). Furthermore, we observed eight homozygote haplotypes in the MS cases that were non-existent in the controls ($p<0.0003$, OR=20.27, CI 2.50-163.8). For the first time, our data highlight the CAV1 upstream purine complex as a novel susceptibility genomic locus in the pathophysiology of MS. Of utmost importance, the region has been conserved across species, including mouse, guinea pig, rhesus macaque, and human. The functional effect of this region remains to be clarified in the future studies.

P08.37 MMP 9 sequence variants and serum level correlations in Myocardial Infarction

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Background: Myocardial Infarction (MI) is a common, multifactorial disorder and studies have revealed a 5 to 7 fold increased risk to MI associated death in first degree relatives of the probands. To date a large number of genes are implicated in MI of which Matrix metalloproteinases (MMPs) represents a potential candidate gene system in the cardiac remodeling and Heart Failure.

Objective: The present study aims to ascertain the association between potentially functional variants of the MMP-9 gene promoter and correlate the same to the serum levels of MMP-9 in the susceptibility to myocardial infarction and to identify the asymptomatic first degree relatives at risk to MI on family screening.

Methods: The study comprises 150 individuals which include 50 cases of angiographically recorded MI, 50 first degree relatives of the patients and 50 age and sex matched healthy controls. Genotyping of MMP 9 is based on PCR-RFLP using restriction enzyme *sph*1 and serum levels were estimated by ELISA using commercial antibody.

Results & Conclusions: The frequencies of the MMP 9 genotype were significantly varied among the three groups of study and the levels seem to be correlated with specific genotypes. Hence it is suggested that MMP-9 plays an important role in the onset and prognosis of myocardial infarction and helps in the diagnosis of asymptomatic and pre symptomatic individuals in screening.

P08.38 A stochastic model for DNA instability in the inherited neuromuscular disease myotonic dystrophy

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Myotonic dystrophy type 1 (DM1) is caused by inheriting an expanded unstable DNA CTG repeat. Generally, an increase between generations causes a decreasing age of onset, whilst an increase in the number of repeats throughout the lifetime of a patient contributes toward the progressive nature of the symptoms. We are interested in modeling mathematically the evolution of repeat length through the lifetime of patients as this has potential for improving prognostic information as well as providing a deeper understanding of the underlying biological process.

Diagnosis of DM1 is usually confirmed by establishing CTG repeat length but the interpretation of this length is compromised by not knowing the inherited allele length and levels of DNA instability which vary among individuals due to environmental and genetic factors.

Quantitative data, collected from small pool PCR analysis of allele length in blood cells from DM1 patients reveals the variation within and between. This rich source of data opens up the possibility of modeling with a stochastic process. By calibrating a new continuous-time, discrete-state stochastic model to this data, we infer the unknown inherited allele length for each patient and quantify the mutational force which has led to their observed distribution of alleles.

We show that these quantitative traits improve prognostic information for age of onset and could be used as biomarkers to identify trans-acting genetic, epigenetic or environmental effects. We conjecture that these trans-acting genetic modifiers also apply in the general population where they will affect ageing, cancer, inherited disease and human genetic variation.

P08.39 OTOF (DFNB9) mutations are very rare in autosomal recessive Non-syndromic Hearing Loss in Iranian population

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Hearing impairment is the most common genetic sensory defect in humans worldwide. It is estimated that profound hearing loss occurs in 4 out of every 10,000 children. About 70% of hereditary SNHL is non-syndromic (DFN) and at least 80% of these cases have autosomal recessive (DFNB) inheritance. To date, genetic studies have shown

that mutations in 79 loci and 28 genes are associated with DFNB. Mutations in *OTOF* gene are a cause of neurosensory non-syndromic recessive deafness, DFNB9. *OTOF* gene contains 48 exons which encodes a transmembrane protein, otoferlin. Several mutations in this gene have been found in Lebanese, Pakistani, Turkish, Colombian and Spanish families. Recently we observed an Iranian ARNHL family which showed linkage to *otof* gene. Mutation detection of this gene revealed a missense mutation in exon 27, so we decided to screen our population for this gene.

Ninty one ARNHL families with two or more affected individuals originated from different ethnic groups of Iran were selected for this study. All the families were subjected to homozygosity mapping using flanking STR markers of *OTOF* gene. After screening all the families, no linkage was established. So in comparison with other countries such as our neighboring country, Pakistan, *OTOF* mutations are very rare in Iran.

P08.40 Common polymorphism in Per3 gene, dietary composition and time structure of energy intake in the Central-European Caucasian population

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Introduction: Recent studies have demonstrated a tight relationship between circadian rhythmicity and the development of obesity and its related traits. In this study, we investigated possible associations of the VNTR polymorphism in the Per3 gene with obesity-related anthropometric traits and behavioral patterns.

Methods: The total of 330 volunteers of various BMI and age distribution originating from the Czech Caucasian population were enrolled in this study in order to evaluate the relationship of VNTR Per3 with anthropometric parameters related to obesity and dietary composition and time patterns of food intake (based on 7-day native food records). The allele-specific PCR-based method was used to determine the VNTR Per3 genotypes.

Results: No differences were observed in genotype or allele frequencies between the obese cases ($BMI \geq 30$) and the non-obese individuals ($BMI < 30$) ($pg = 0.92$; $pa = 0.86$). The VNTR Per3 was further associated with the energy value of lunch on average working day ($p < 0.02$). When analyzing the dietary composition, the VNTR Per3 polymorphism was associated with the total daily energy derived from carbohydrates ($p < 0.01$) and fat ($p < 0.02$), whereas the 55 homozygotes expressed the highest daily intake of carbohydrates and fat, respectively. No association with aberrant behavioral patterns - extreme snacking or extreme portion sizes was observed.

Discussion: This is the first study to investigate the relationship between VNTR polymorphism in Per3 gene and behavioral patterns of the food intake in humans; VNTR seems to influence rather the qualitative dietary structure than the time patterns of food intake.

P08.41 Association study between G-protein-coupled receptor kinase 5 gene and Parkinson's disease

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The major component of Lewy Bodies, the pathological hallmark of Parkinson's disease is α -synuclein, most prominently phosphorylated at serine 129. It is not clear which is exactly the contribution of members of G-protein-coupled receptor kinase family (GRKs). However, it has been shown that the GRK5 phosphorylates Ser 129 in vitro and enhances α -synuclein toxicity. Moreover, a genetic association study performed in an Japanese population revealed haplotypic association of the GRK5 gene with susceptibility to sporadic PD. We aimed at investigating whether four polymorphisms within the GRK5 gene (rs871196, rs2420616, rs7069375, rs4752293) could represent a risk factor for sporadic PD in Southern Italy. We genotyped 446 patients with PD and 450 controls for these markers and we did not find any significant association with the disease at allelic, genotypic and hap-

lotypic level. Our results indicate that the GRK5 gene does not confer risk to sporadic PD in our sample from southern Italy.

P08.42 Per3 VNTR polymorphism and chronic heart failure

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Introduction: The human homologue of *Drosophila* clock gene PERIOD3 (PER3) gene has been recently demonstrated to affect circadian expression of various proteins in variety of tissues including heart. Alterations in the circadian patterns of variety of circulatory functions are frequently observed in cardiovascular diseases. No studies of Per3 gene on chronic heart failure (CHF) patients have been conducted so far, therefore the aim of the study was to investigate the effect of variable number tandem repeat (VNTR) polymorphism in the coding region of the circadian clock Per3 gene on CHF.

Methods: This case-control study comprised a total of 371 patients of Caucasian origin with chronic heart failure (functional classes NYHA II-IV, ejection fraction (EF) < 40%) and 332 healthy controls of similar age and gender distribution. The study subjects were genotyped for Per3 VNTR polymorphism using an allele-specific PCR-based methodology.

Results: No significant differences in genotype or allele frequencies of Per3 VNTR were observed when comparing CHF cases and control ($p_g = 0.30$, $p_a = 0.52$); moreover, no significant differences were observed when comparing CHF cases according to their etiology ($p_g = 0.87$, $p_a = 0.91$). In the multivariate regression modeling, no predictive function of VNTR Per3 polymorphism on ejection fraction or NYHA class, hyperlipidaemia or type II diabetes risk was observed.

Discussion: Based on the results of the presented study, we do not consider the Per3 VNTR polymorphism a major risk factor for chronic heart failure or a factor modulating severity of the CHF in the investigated Caucasian population.

P08.43 Evaluating the PHARMAchip™ array: Pharmacogenetic profile assessment in Spanish control population.

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Objectives: 1) To determine the analytical and clinical validity of PHARMAchip array before its routine use in clinical practice; 2) To investigate the frequencies of important allelic variants in 36 genes involved in pharmacogenetics in Spanish control population.

Methods: 437 unrelated Spanish controls that were genotyped using the PHARMAchip. Analytical validity was determined for CYP2D6 and SLC6A4 genes and results obtained by the array were compared to PCR-based methods. Clinical validity was also determined for CYP2D6. Allelic frequencies were calculated and statistically analysed.

Results: Sensitivity (S) and specificity (SP) values of the array ranged from 98-100% for CYP2D6 alleles. For SLC6A4, S and SP were of 96.5 % and 97.9%, respectively. For the diagnosis of poor metabolizer status, S was 87.5% and SP 98.6%. For ultra-rapid metabolizer status, both values were 100%. All genes were in Hardy-Weinberg equilibrium, with the exception of ADRB2. In terms of metabolizers status, the percentages of poor, intermediate, extensive and ultrarapid metabolizers were similar to those described in other Caucasian populations.

Conclusions: To best of our knowledge, this population-based study shows for the first time the allelic distribution of 90 pharmacogenetic variants among Spanish population. The availability of this genetic information, and the functional significance of these polymorphisms affecting drug metabolism, should facilitate their application to pharmacogenetic profiling. PHARMAchip is an accurate, rapid and updatable tool for assessing an individual's genetic background in relation to drug

response. Nevertheless, cost-effectiveness should be further analyzed before its implementation in clinical practice.

P08.44 Search of associations of *IL12A*, *IL12B*, *IL12RB1* polymorphisms with tuberculosis, bronchial asthma and ischemic heart disease

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Polymorphisms in *IL12A* (rs568408), *IL12B* (rs32122 and rs321227), *IL12RB1* (rs3746190 and rs11575926) were studied in the population sample (Russians, Tomsk) and in three samples of patients collected in the same population: (1) ischemic heart disease combined with hypertension, (2) tuberculosis, (3) bronchial asthma. For the study, DNA collections of the Institute of Medical Genetics were used. For all SNPs, no deviation from Hardy-Weinberg equilibrium was detected. Comparison of patient groups with population sample revealed an association of *IL12A* (rs568408, allele G and genotype GG) with bronchial asthma. For allele G carriers, $OR=1.65$ ($I=1.01-2.7$; $\chi^2=4.01$ ($p<0.05$)). For genotype GG, $OR=2.00$ ($I=1.12-3.58$; $\chi^2=5.56$ ($p<0.05$)). Comparison of different patients groups has shown differences in genotypes frequencies in the same locus between asthma group and ischemic heart disease ($\chi^2=6.65$, $p<0.05$), as well as between asthma and tuberculosis ($\chi^2=8.006$, $p<0.05$). There were no significant associations for individual SNPs in *IL12RB1* gene, but the genotypes combinations were different in cases and controls. In particular, we found that combination of genotype CC rs3746190 with AG rs11575926 in *IL12RB1* is protective for all three diseases studied: for ischemic disease ($OR=0.08$ ($I=0.00-0.62$; $\chi^2 = 7.32$ ($p<0.05$))), tuberculosis ($OR=0.10$ ($I=0.01-0.54$; $\chi^2=9.37$ ($p<0.005$))), and asthma ($OR=0.23$ ($I=0.05-0.97$; $\chi^2=4.04$ ($p<0.05$))). So, we have shown involvement of SNPs in *IL12A* (rs568408) and *IL12RB1* (rs3746190 combined with rs11575926) into pathological phenotype in different diseases.

P08.45 The effect of peroxisome proliferator - activated receptors polymorphisms on the physical performance of Lithuanian athletes

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Variation in genes involved in lipid, glucose metabolism and energy homeostasis is expected to have a role in the physical performance. Although the polymorphisms of *PPARA* intron 7 G/C (rs4253778) and *PPARG* Pro12Ala (rs1801282) have been suggested to influence variation in skeletal muscle function, data has been conflicting. We have investigated the genotype and allele distribution of *PPARA* and *PPARG* polymorphisms in Lithuanian athletes ($N=193$) and controls ($N=250$). The athletes were stratified into three groups: endurance ($N=77$), power ($N=51$) and mixed ($N=65$). There were 43 'elite', 52 'sub-elite', 98 'non-elite' athletes. Genotyping was performed by PCR-RFLP. The results of the present study imply that the *PPARA* C allele is more common in the athlete group than in the general population of Lithuania (G/C 77.5/22.5% vs G/C 82.8/17.2%, $P=0.046$). Genotype frequency did not differ between all athletes and controls, but differed significantly between the 'elite' athlete group and the controls (GG/GC/CC: 51.2/37.2/11.6% vs 69.2/27.2/3.6%; $P = 0.018$). Moreover, the frequency of CC genotype was higher in elite athletes (11.6%) than sub-elite (7.7%) and non-elite (2%) athletes. No significant differences between the athletes and controls were found according to the allele and genotype frequencies of the *PPARG* polymorphism. The present findings suggest that there is an association between *PPARA* G/C polymorphism and physical performance in Lithuanian athletes. Conversely, the importance of *PPARG* polymorphism in athletic performance is not straightforward and can be masked by other genetic and non-genetic factors as no association was found with athletic success.

P08.46 A Common Polymorphism in the Cannabinoid Receptor 1 (CNR1) Gene is Associated with Pre-eclampsia in the Central-European Caucasian Population

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Introduction: Endocannabinoids have been recently suggested to play a fundamental role in early placental development. The aim of the study was to investigate associations of three SNPs in the endocannabinoid type I receptor gene (CNR1) (rs1049353, rs12720071 and rs806368) and their haplotypes with pre-eclampsia, a severe pregnancy-associated condition characterized by abnormal development and remodeling of spiral decidual arteries.

Methods: The case-control study comprised a total of 115 pre-eclamptic women and 145 healthy pregnant controls, all originating from the Czech Caucasian population. Using PCR-based methods, we tested rs1049353, rs12720071 and rs806368 in CNR1 gene and haplotypes were constructed.

Results: Significant difference in genotype distributions of rs806368 ($p < 10^{-5}$) was observed when comparing the cases and the controls; the cases presenting with significantly higher proportion of heterozygotes. In the regression modeling, the rs806368 served as a predictor for pre-eclampsia development ($\beta = 0.15$; $p = 0.04$). Haplotype analysis revealed presence of 4 common haplotypes; the AAC haplotype being less frequent in pre-eclamptic cases compared to the controls ($p < 0.008$), whereas the prediction role of AAC haplotype for pre-eclampsia onset ($\beta = -0.18$; $p = 0.03$) was confirmed further by analysis of regression model. None of investigated polymorphisms or haplotypes was associated with IUGR development.

Discussion: This is the first study focusing on relationship between SNPs in CNR1 gene and pre-eclampsia risk. Although limited by a relatively small sample size, the study clearly indicates that rs806368 in the CNR1 gene may act as attractive susceptibility marker for pre-eclampsia in humans.

P08.47 Novel *IL31RA* gene mutation and haplotype block structure of the *OSMR* mutation region in familial primary cutaneous amyloidosis

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Primary cutaneous amyloidosis (PCA) is an itchy skin disorder associated with amyloid deposits in the superficial dermis. The disease is commonly found in Southeast Asia and South America. Autosomal dominant PCA has been mapped previously to 5p13.1-q11.2 and 2 pathogenic missense mutations in the *OSMR* gene, which encodes the interleukin-6 family cytokine receptor oncostatin M receptor beta (*OSMR*β), were reported. We investigated 29 Taiwanese pedigrees with PCA and previously reported 10 had heterozygous missense mutations in *OSMR*: p.D647V (1 family), p.P694L (6 families), and p.K697T (3 families). The mutation p.P694L was found to be associated with the same haplotype in 5 of 6 families with PCA. Of the other 19 pedigrees that lacked *OSMR* pathology, 8 mapped to the same locus on chromosome 5, which also contains the genes for 3 other interleukin-6 family cytokine receptors, including interleukin-31 receptor A (*IL31RA*), which can form a heterodimeric receptor with *OSMR*β through interleukin-31 signaling. In one family, we identified a point mutation in the *IL31RA* gene, c.1562C>T, that results in a missense mutation, p.S521F, which is also sited within a fibronectin type III-like repeat domain as observed in the *OSMR* mutations. PCA is a genetically heterogeneous disorder but our study shows that it can be caused by mutations in 2 biologically associated cytokine receptor genes located on chromosome 5. The identification of *OSMR* and *IL31RA* gene pathology provides an explanation of the high prevalence of PCA in Taiwan and new insight into disease pathophysiology.

P08.48 The *PTPN22* Gene Influences Genetic Predisposition to Ulcerative Colitis and Crohn's Disease.

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The *PTPN22* gene, encoding the lymphoid_tyrosine_phosphatase-*LYP*, is an important risk factor for human autoimmunity. Recently a novel polymorphism (R263Q;rs33996649) within the catalytic domain of *LYP* has been associated with reduced risk of SLE. And the R620W;rs2476601 *LYP* variant is a risk factor of several autoimmune diseases. Based on this, we evaluated the role of both polymorphisms in ulcerative colitis (UC) and Cohn's disease (CD).

A total of 2176 CD patients, 1764 UC patients and 2950 healthy controls, from an initial case-control set of Spanish Caucasian ancestry and two independent cohorts of European-ancestry (Dutch and New Zealand) were included in the study. Genotyping was performed using TaqMan® allelic discrimination assay for the rs33996649 and rs2476601 polymorphisms in the *PTPN22* gene. Meta-analysis was performed to test the overall effect of each polymorphism in UC and CD (Including for the R620W: 5305UC, 7081CD, 9502 healthy controls and for R263Q: 2085UC, 2434CD, 3102 healthy controls).

We found that the R263Q *PTPN22* polymorphism is significantly associated with UC in the Spanish population ($P=0.017$, $OR=0.64, 95\% CI=0.43-0.94$) and the meta-analysis confirmed this association ($P=0.007$ pooled, $OR=0.69, 95\% CI=0.52-0.90$). Although, the R263Q polymorphism is not significant associated to CD ($P=0.29$ pooled, $OR=1.13, 95\% CI=0.89-1.43$), we found through the meta-analysis, that the C allele of the R620W *PTPN22* variant is a risk factor to develop CD ($P=0.001$ 1 pooled, $OR=1.29, 95\% CI=1.13-1.46$) but is not for UC ($P=0.08$ pooled, $OR=1.09, 95\% CI=0.95-1.25$) in the Caucasian population.

Our data suggest that the A allele of the R263Q *PTPN22* polymorphism reduced the risk of UC. While, the C allele of the R620W *PTPN22* polymorphism is a risk factor to CD.

P08.49 Development of real-time PCR assay and comparison with RFLP genotyping of 49A/G *CTLA-4*

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The cytotoxic T-lymphocyte antigen-4 (CTLA-4) has a role in controlling immune response delivering a negative signal to T-cell activation. A single nucleotide polymorphism at position 49 in the exon 1 of the *CTLA-4* gene (49A/G) has been associated with several autoimmune diseases. In order to analyze the 49A/G polymorphism a conventional polymerase chain reaction (PCR) followed by the restriction fragment length polymorphism method (RFLP) has been described. Although RFLP has widely been accepted as suitable method, more automated and faster genotyping method might be needed for routine use and population studies. In our previously described study the genotyping of 49A/G polymorphism by RFLP using *Bse*XI enzyme was carried out. Here are reported data on the design of primers and MGB-probes used in the real-time PCR assay to detect the 49A/G polymorphism. A comparison of the used RFLP and the new real-time PCR assay has been done on 45 samples. Four results were found to be discordant between the two methods. Three samples defined as heterozygotes (AG) by the RFLP method were identified as mutant homozygotes (GG) using the real-time PCR assay. In one case RFLP-defined wild type homozygote (AA) was identified as heterozygote. Cause of these discordant results could be incomplete enzyme restriction in RFLP which is avoided in real-time PCR assay. In addition, using real-time PCR method a possible cross-contamination is minimized. Therefore, it could be concluded that the developed real-time PCR assay gives

more accurate results than RFLP and is more appropriate for 49A/G CTLA-4 genotyping.

P08.50 Identification of a novel locus for autosomal dominant Restless Legs Syndrome in an Irish Pedigree

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Introduction: Restless Legs Syndrome (RLS) is a common neurological sleep-related disorder, which affects 5-10% of the general population. The disorder is characterised by an irresistible urge to move the lower limbs associated with unpleasant sensations. Symptoms present while at rest, generally in the evening, and are relieved, at least partially and temporarily, by movement. The condition is predominantly an autosomal dominant disorder. Molecular genetic approaches have identified six distinct genomic regions. No specific gene within these loci has been reported thus far. Association mapping has highlighted a further five areas of interest.

Aim: The goal of this research was to map and identify the gene responsible for the syndrome in a newly identified autosomal dominant Irish RLS family (RLS3002).

Method: Eighteen members of the RLS3002 family participated in the study; eleven affected and seven unaffected members. All known RLS loci and associated regions were examined for linkage. A genome wide linkage analysis scan was then conducted.

Result: Linkage was excluded from all published RLS loci. The genome-wide scan identified a region of linkage with a maximum LOD score of 3.59, at a theta value of 0. A genetic region which corresponds to 2.5 Mb was defined by haplotype analysis. A number of candidate genes have been identified and are the subject of further study.

Conclusion: We have successfully identified a novel locus for autosomal dominant Restless Legs Syndrome, which will enable us to attempt to identify the causative gene in this family.

P08.51 Pharmacogenetics of stroke (Geno-tPA study): genetic background predicts hemorrhagic transformation after thrombolytic therapy in ischemic stroke

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Background: t-PA is the unique treatment for acute ischemic stroke management. Despite its global beneficial effect, 20-30% of t-PA treated patients suffer hemorrhagic transformations (HT).

Methods: Two cohorts of 540 and 360 stroke t-PA treated subjects were analyzed. 162 SNP were genotyped in both cohorts using SNPlex technology. Results robustness was assessed by bootstrap analysis. Baseline protein and mRNA levels of associated genes were determined in 140 individuals. Finally, a predictive model was generated with clinical and genetic data.

Results: 9 SNP were independently associated with HT appearance in the first 540-cohort. One of them, located in a gene related to blood brain barrier permeability, resisted Bonferroni correction and was validated in the replication cohort using either dominant model or additive model [Table 1]. Furthermore, it was consistently associated in bootstrap analysis. This variant correlated with plasmatic protein levels ($p=0.016$) but not with baseline mRNA levels ($p=0.669$). Another SNP was associated in bootstrap analysis, which was in the threshold of significance [Table 1]. This SNP influenced protein activity ($p=0.004$) and was associated with intrahospitalary death ($p900=0.00048$).

The predictive model allowed us to predict HT up to 50% occurrence with higher predictive capacity than clinical data alone.

Conclusion: genetic background is underlying HT appearance after thrombolytic therapy and might be a useful tool to predict hemorrhagic complications in acute stroke management.

| | AA540 | B-carriers540 | p540 | AA360 | B-carriers360 | p360 | |
|-------|-------------|---------------|----------|-------------|---------------|--------|-----------|
| SNP_1 | 30.5% | 15% | 8.8*10-5 | 25.7% | 15.5% | 0.02 | |
| SNP_2 | 25% | 18.1% | 0.102 | 24.9% | 12.9% | 0.011 | |
| | A-allele540 | B-allele540 | p540 | A-allele360 | B-allele360 | p360 | Bootstrap |
| SNP_1 | 25.8% | 14.7% | 0.0002 | 23.1% | 14.6% | 0.0136 | 964/1000 |
| SNP_2 | 23.9% | 17.3% | 0.074 | 23% | 11.5% | 0.004 | 722/1000 |

P08.52 -1082A genetic variant of interleukin-10 gene promoter linked to schizophrenia in a Spanish schizophrenic population

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Introduction: Schizophrenia aetiology is predominantly genetic with up to 80% heritability presumably caused by the interaction of a cluster of genes, each contributing with small increase in the global risk. Massive information is emerging from association studies that give rise to multiple candidate genes no consistently replicated. The aim of this study is to analyze the relationship of variants in genes previously linked to schizophrenia, namely SLC6A4, HTR2A, DRD3, COMT, GRIN2B, TNF α and IL-10.

Subjects and methods: 261 unrelated schizophrenic patients and 437 controls were genotyped for the genetic variants selected, through PHARMAChip, a Pharmacogenetic tool with polymorphisms involved in the therapeutic outcome. Pearson's χ^2 test, Bonferroni correction and Hardy-Weinberg equilibrium were used to assess genetic association. Results: IL-10 -1082A allele and A/A genotype were significantly increased in schizophrenic patients compared to controls ($\chi^2=7.75$; $p=0.005$ and $\chi^2=11.57$; $p=0.0007$ respectively). IL-10 genotypes did not deviate from Hardy-Weinberg equilibrium.

Discussion: Three single nucleotide polymorphisms have been described in the promoter of the IL-10 gene, which define three haplotypes in Caucasian populations. These haplotypes are known to influence IL-10 production with high, intermediate and low producing haplotypes. High producing allele -1082G and haplotype have been linked to schizophrenia. We found the low producing allele -1082A associated to schizophrenia. Thus, haplotype distribution investigation is needed to elucidate the role of interleukin 10 in schizophrenia. Insufficient sample size or exclusion of causative role could be the explanation for the lack of association found in the remaining genes

P08.53 Association of G72/G30 polymorphisms with schizophrenia in the populations from Bashkortostan (Russia)

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Recently, the nested genes G72 and G30 on chromosome 13q32-q33 have been implicated in the etiology of schizophrenia. We hypothesized that polymorphic changes in this gene region might be associated with schizophrenia in populations from Bashkortostan (Russia). We investigated four SNPs (rs2391191, rs3918342, rs978714 and rs1341402) in 351 patients (131 Russians, 112 Tatars and 108 Bashkirs) with schizophrenia and 423 control subjects (115 Russians, 168 Tatars and 140 Bashkirs). Case-control comparisons were based on association analysis, linkage disequilibrium (LD) and haplotype frequency estimations. The T/T genotype (rs3918342) associated with an increased risk (OR = 1.87; CI 95% 1.08 - 3.24) of schizophrenia in Russians patients. Maximum likelihood analysis of haplotype distribution demonstrated the presence of a linkage disequilibrium between the two loci rs2391191 and rs1341402 in subjects of the Bashkirs ($D'=52\%$), Russians ($D'=75\%$), Tatars ($D'=59\%$) descent. Further analysis showed overrepresentation of the CG haplotype ($p=0.081$) among Tatar patients compared to control. Conclusions: Our findings suggest that the genes G72/G30 may be associated with schizophrenia; however, the effect is influenced by ethnicity.

P08.54 Evidence of the future synpolydactyly genetic heterogeneity in SPD1 locus

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Synpolydactyly (SPD) is a rare limb deformity showing a distinctive combination of syndactyly and polydactyly. SPD is characterized by webbing between 3/4 fingers and between 4/5 toes. Three loci have been identified at chromosomes 2q31 (SPD1), 22q13.31 (SPD2) and 14q11.2-q12 (SPD3).

The five generation Russian family was ascertained. A detailed pedigree was constructed and 15 subjects were physically examined. Eight subjects were found to be affected. Five members has syndactyly of the third and fourth fingers both hands, one member of one hand and two members has syndactyly of the third and fourth hands fingers and polydactyly of the fifth toe in the feet.

To test the candidate loci for linkage with the phenotype segregating in the Russian family, microsatellite markers from region 2q31-q32 (SPD1: D2S1776, D2S326, D2S2314, D2S324, D2S311, D2S164, D2S2250 and intragenic CA repeat of HOXD13 intron 1); from region 22q13 (SPD2: D2S1141, D2S928, D2S274) were selected. Two microsatellite markers flanking the SPD1 candidate interval (D2S2314, D2S324) and intragenic CA repeat revealed significantly high LOD scores ($Z_{\text{max}} \geq 2$) indicating a strong evidence of linkage between this locus and the phenotype segregating in the Russian family. However at research of all coding, not coding and regulatory regions of HOXD13 gene it was not revealed any nucleotides substitutions except rs72923424. Polyalanine repeat expansion mutations were not found too.

The received results can testify about future genetic heterogeneity of synpolydactyly in SPD1 locus and possible involving of others locus 2q31-q32 HOX genes to SPD pathogenesis.

P08.55 Association of type 1 diabetes to the MHC2TA gene and genotype frequencies among controls with age

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The MHC2TA gene, which is crucial in expression of MHCII molecules, has been reported to associate to a wide range of diseases like Rheumatoid Arthritis (RA), Multiple Sclerosis (MS) and Myocardial Infarction (MI). These results have been replicated with varying results depending partly on which control group was used. Linkage to chromosome 16p13 where MHC2TA is situated has been reported to type 1 diabetes (T1D). Here we test whether the gene is associated to T1D and MS with correction for age and if there is a change in genotype frequency depending on age in the MHC2TA gene among the controls.

Several markers in the MHC2TA gene were genotyped using mainly Taqman allelic discrimination in two large materials of MS and T1D patients. Also >10000 controls from studies of RA, MS, T1D, MI and Alzheimer's disease (AD) was typed for 3 SNP markers. The controls were divided into 5 years intervals with respect to age at disease onset/sampling. Chi-squared test was used to detect significant association; Chi-squared Test for Trend in Proportions was used to detect the overall trend in genotype over age, and logistic regression was used to control for age-differences.

The results shows significant association to T1D (RS11074930 p=0.03, RS11074932 p=0.008, RS3087456 p=0.02) but not to MS for the 3 markers when correcting for age, also there is a significant difference in the genotype distribution for all 3 markers with respect to age among controls. This could have several causes that need to be further investigated.

P08.56 The efficiency of multiple splitting strategy for linkage analysis

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The large pedigrees from isolated populations are very informative for multipoint linkage analysis of complex traits. However, to analyze them by using existing programs for linkage analysis, it is necessary to split the pedigree into non-overlapping fragments of the suitable size. This splitting can be done in various ways to produce multiple sub-pedigrees sets with quite different characteristics. Each of these sets would be informative to detect linkage signals on different chromosomal regions. We proposed to use several splittings for genome-wide linkage maximizing LODscore on all the splittings for each marker. The main problem which arises due to multiple testing is generating a correct genome-wide significance threshold for linkage result. To assess the empirical type I error and the efficiency of our strategy, we performed a simulation study of a quantitative trait. We split the complex pedigree including 1500 members by use programs Jenti (Falchi & Fuchsberger, 2008), PedCut (Liu et al., 2008) and PedStr (Kirichenko et al., 2009) into three sub-pedigrees sets. The threshold corresponding to significance level depended from splitting and varied within 5%. The same threshold for maxLODscores increased by 14 % of average threshold. However, power of the analysis was retained and the detection of QTL position became more accurate. We believe the use of several splittings instead of one, is quite effective strategy for search of genes of complex traits.

P08.57 Vitamin D receptor genetic polymorphism and bone mineral density in Iranian postmenopausal women

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Objective: Osteoporosis is one of the most important medical problems facing the aging population especially postmenopausal women. It is a common disease with a strong genetic component. Polymorphism of the vitamin D receptor (VDR) gene has been reported to play a major role in variations for genetic regulation of bone mass. To clearance of its role Bsm1 polymorphism were studied in Iranian postmenopausal women.

Methods: 128 women from Tehran Lipid and Glucose Study (TLGS) study (53 postmenopausal, 75 premenopausal) were selected who underwent BMD testing, serum 25(OH) D, calcium, phosphorus and bone mass were measured, they had genotyping for the VDR alleles using polymerase chain reaction methods and restriction fragment length polymorphism with Bsm1 based on the present (b) and the absent (B).

Results: In current study the respective frequencies of VDR genotype were BB 18%, Bb 52% and 30% bb. After adjusting the influence of age and menopausal status, the analysis of co-variance (ANCOVA) revealed that the presence of the bb genotype increases the BMD in premenopausal individuals (Femoral neck, p 0.005; Trochanteric, p 0.047; Total femoral, p 0.051; Lumbar spine (L1-L4), p 0.007).

Conclusion: It was found that there is significantly association between VDR gene polymorphism with bone mineral density in different menopausal situation. These finding suggest that the ethnic diversity in VDR genotype frequencies, the association between the genotypes and bone mass might be in part responsible for the ethnic differences in bone mass.

P08.58 Identity-by-descent probability estimation for multiple SNP markers

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Availability of dense SNP maps has opened new possibilities for identity-by-descent (IBD) calculations. Development of the new methods for large pedigrees is actual because large number of individual genome sequences will be available soon. Application of recursive methods of approximate IBD computation is the most acceptable for the large complex pedigrees and large number of analysed markers. One of such approaches had been proposed by Wang et al. (1995) for arbitrary pedigrees with complete marker information. But its application

to the multipoint genotype data is not expedient, because in this case many related pairs may be non-informative. The problem was solved by recursive multi-point approach proposed by Pong-Wong et al. (2001). This approach permits to obtain effective IBD probabilities by genotypes of informative flanking loci due to specifying transmission of alleles of non-informative locus from parents to offspring but only with individuals whose parents and grandparents were genotyped. To find effective IBD estimates with all individuals of large complex pedigree with ungenotyped generations, we develop a universal approach combining these recursive methods, which expands limits of their application. The approach makes it possible to compute quickly and sufficiently exactly IBD estimates using any multipoint genotype data.

J08.1 A study of anthropometric trait in Korean Twin cohort

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We surveyed the 1520 respondents drawn from the Korean Genome and Epidemiology Study (KoGES), which is an ongoing prospective twin cohort study and also genotyped 1,861 samples from the Korean Genome and Project (KoGAP). This study is to assess the impact of their influence including single nucleotide polymorphisms from Korean Twin. Genomic DNA from peripheral blood mononuclear cells was used for this study. We recruited twin pairs first using a population-based twin cohort in Korea. These twins were of Korean ancestry, to analyze their genome association within 1 ethnicity. There were 1298 individuals from 374 twin pairs who have clinical, epidemiological and genotyping data, including 224 monozygotic and 169 dizygotic individuals. We performed a Genome-wide association study, typing cases and controls on a single platform using the Affymetrix Genome-Wide Human SNP array chip (Affymetrix 6.0 SNP chip). Genotype calls were determined by Birdseed algorithm.

For clinical, epidemiological and genotype data related to anthropometry in Korean twin cohort data, we tried to analyze heritable and significances. Especially, we showed the differences of metabolic traits using generalized estimating equations (GEEs) were performed on age- and sex-adjusted data.

J08.2 Genetic component in polyaetiology models of chronic generalaized periodontitis with an aggressive current.

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¹MGMSU, Moscow, Russian Federation, ²RCMG, Moscow, Russian Federation. Prevalence of inflammatory diseases periodontitis in age group of 35-44 years on the world makes 94.3 %. In disease development periodontitis assume presence genetic components. The basic directions of search candidate genes were made among markers of infringement of a bone metabolism. On the basis of a clinical picture and the given laboratory analyses (raised level PTH and decrease CALC), for studying have been chosen polymorphisms genes CALCR, COLIA1 and PTHR1, earlier defined as hereditary factors of decrease bone mineral density. Research objective was to definite genetic components of chronic generalaized periodontitis with an aggressive current (AgP). Research of polymorphic variants COLIA1 (rs1800012) and CALCR (rs1801197), was spent by a Multiplex Ligation-dependent Probe Amplification (MLPA), for PTHR1 research has been chosen VNTR from promoter region, genotypes was carried out by a amplification fragments lengths polymorphism (AFLP). The research was made on DNA samples of 151 person of both sexes which have divided into 3 groups. 1st group included patients with AgP, middle age 38.9±5.7, 2nd group included patients with a system osteoporosis (OP) middle age 68.2±5.4, 3rd group has made casual populations sample (CPS), middle age 45.7±5.3.

| Groups | AgP | | OP | | CPS | |
|--------|------------------|------|------|------|------|------|
| | Alleles CALCR | N=43 | % | N=40 | % | N=78 |
| T | 61 | 0.71 | 53 | 0.68 | 120 | 0.77 |
| C | 25 | 0.29 | 23 | 0.32 | 36 | 0.23 |
| COL1A1 | N=43 | % | N=40 | % | N=77 | % |
| G | 44 | 0.51 | 68 | 0.76 | 124 | 0.82 |
| T | 42 | 0.49 | 8 | 0.24 | 28 | 0.18 |
| PTHR1 | N=43 | % | N=40 | % | N=82 | % |
| 5 | 65 | 0.76 | 56 | 0.76 | 106 | 0.67 |
| 6 | 21 | 0.24 | 18 | 0.24 | 51 | 0.32 |
| 9 | 0 | 0 | 0 | 0 | 1 | 0.01 |

The results of researches have shown authentic distinction on COL1A1 in group AgP in comparison with CPS ($p \leq 0.05$).

J08.3 Association between +12545GT CollA1 genotypes with different stages of caries in children with chronic gastroduodenitis.

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Collagen I type is one of structural proteins of connective tissue. The changes of collagen structure associated with any connective tissue disorders, with symptoms of gastrointestinal involvement.

The aim of our study were children with caries and chronic gastroduodenitis. Methods: We included 70 children aged 12-17 years, with different stages of caries and chronic gastroduodenitis with morphological approve. Morphological changes ranged from different degree of fibrosis of mucous stomach membrane: 27 with intensive, 26 with moderate and 16 without fibrosis. Dental examination was revealed different stages of caries. Molecular testing of +12545GT polymorphism of CollA1 gene was carried out by RFLP.

Results: ss genotype of CollA1 gene was revealed in 5 patients with decompensate caries (7.5%). Ss genotype was determined in 53.7% with decompensate and in 28.3% with compensate caries ($p < 0.05$) and in 11.9% of children without caries $p < 0.05$). Frequency of SS genotype decreased relatively to degree of caries ($p < 0.05$).

Children with intensive stomach mucous membrane fibrosis have rare SS genotype compare with children without fibrosis (33.3% compare 72%) and frequently Ss (42.6% compare 28%) and ss genotypes (24% compare 0%), chi-square=20.11, $p=0.0004$, $C=0.58$. We have no detected significant differences in genotypes distribution of CollA1 genotypes between patients with moderate fibrosis and without it.

Conclusion: children with Ss and ss genotypes more frequently have decompensate caries (53.7% and 7.5%) and also have more significant fibrosis changes in stomach mucous membrane (Ss - 42.6% and ss - 24%). Children with decompensate stages of caries need in wide endoscope and morphological examination.

J08.4 Association between a polymorphism in the G-protein $\beta 3$ subunit gene and unipolar depression

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A dysfunction of the serotonergic system can lead to development of unipolar depression (UD). There are 14 different serotonin receptors, 13 of which are G-protein-coupled receptors. The T-allele of the C825T polymorphism of the GNB3 gene coding G-protein $\beta 3$ subunit was found to be associated with the expression of a short splice variant termed G3 β s, a biologically active G β -protein that may be associated with enhanced signal transduction. A total of 230 patients with UD (95 Russians and 123 Tatars) were included in this study. The control group included 357 individuals (83 Russians and 274 Tatars) without a personal/family history of any psychiatric disorders. The C825T polymorphism of the GNB3 gene was analyzed using PCR technique. There were no statistical differences between UD patients and healthy controls in the genotypic and allelic distribution of the GNB3 polymorphism investigated. But we found the tendency to increase *C-allele in

patient group of Tatars ethnicity: $\chi^2 = 3,403$, $P=0,065$.

The research was supported by the Russian Humanitarian Research Fund (the grant number 08-06-00579a).

J08.5 Analysis of interaction between rs2476601 and rs10818488 SNPs in causing risk for rheumatoid arthritis

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Rheumatoid arthritis (RA) is a common autoimmune disease with a complex etiology affecting 1% of the world population. It appears that RA requires the complex interaction of genetic and environmental factors with the immune system, and ultimately in the synovial tissues. The role of genetic elements in determining both the risk of developing RA and the severity of the disease had already been acknowledged. 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 non-receptor 22) gene can be named as the second polymorphism that has been repeatedly reported to be associated with RA. The association of another SNP, rs10818488 located near TRAF1 gene, has been recently picked up by genome wide association studies. So far there is no report investigating the possible association of rs2476601 and rs10818488 SNPs with RA in Iranian population. Therefore the aim of this study is to determine the possible association of these SNPs with RA in Iranian population using family-based (parents-child trios) as well as population-based case-control studies.

In addition to testing for association, analysis of interaction between the two SNPs will be performed on obtained data.

Currently the genotyping of samples are being carried out. The results will be presented at the meeting.

P09 Complex traits and polygenic disorders

P09.001 Genetic risk factors underlying Achilles tendinopathy: The IL-1 β , IL-1RN and IL-6 genes

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An increase in the incidence of overuse injuries such as Achilles tendinopathy (TEN) was noted. A number of extrinsic and intrinsic risk factors have been suggested to be associated with increased risk including genetic elements within the COL5A1, TNC, MMP3, GDF5 genes. The aim was to investigate if functional polymorphisms within genes encoding proteins involved in tendon signalling pathways such as IL-1 β (rs16944 and rs1143267), IL-1RN (rs2234663), IL-6 (rs1800795) were associated with AT in a South Africa (SA) and an Australian (AUS) case-control study.

171 TEN (89 SA and 82 AUS) and 356 asymptomatic control subjects (CON) (158 SA and 198 AUS) were genotyped using RFLP analysis. Chi-squared or Fisher's exact tests were applied (significance $p<0.05$).

The rs16944:TT genotype (OR=3.4, 95% CI 1.2-9.6; $p=0.018$) and rs1143267: C allele (OR=1.7, 95% CI 1.0-2.7; $p=0.048$) in IL-1 β were significantly over represented in the SA male CON group. No differences were noted between the AUS male/female or SA female groups. No associations were observed for IL-RN in either population.

The GG genotype (OR = 2.1, 95% CI 1.2-3.6; $p=0.017$) and G allele (OR = 1.5, 95% CI 1.0-2.2; $p=0.044$) of IL-6 rs1800795 was significantly over represented in AUS TEN, however no differences were noted in the SA groups.

In conclusion, the rs16944:TT and rs1143267:C in IL-1 β are associated with reduced risk of TEN; IL-6 rs1800795:GG and G allele are associated with increased risk of TEN. This provides preliminary evidence for the involvement of the inflammatory pathway in the development of AT.

P09.002 Epistasis between neurochemical gene polymorphisms and risk for ADHD

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A number of genes with function related to synaptic neurochemistry have been associated with Attention-Deficit/Hyperactivity Disorder. However, susceptibility to the development of common psychiatric disorders by single variants acting alone, can so far only explain a small proportion of the heritability of the phenotype. It has been postulated that the unexplained "dark heritability" may at least in part be due to epistatic effects, which may account for the small observed marginal associations, and the difficulties with replication of positive findings. We undertook a comprehensive exploration of pair-wise interactions between genetic variants in 24 candidate genes involved in monoaminergic catabolism, anabolism, release, re-uptake and signal transmission, in a sample of 177 parent-affected child trios using a case-only design. Marker-pairs showing large effects and statistical significance were further explored with a case-pseudocontrol design using conditional logistic regression. We detected a number of interaction odds ratios greater than 4.0, including an interesting correlation between markers in the ADRA1B and DBH genes in affected individuals, and several further interesting, but smaller effects. These effects are no larger than you would expect by chance under the assumption of independence of all pair-wise relations, however independence is unlikely. Furthermore, the size of these effects is of interest and attempts to replicate these results in other samples are anticipated.

P09.003 Clinical validation of age of onset predictor for late-onset Alzheimer's Disease.

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A variable, polyT length mutation within intron 6 of the TOMM40 gene, adjacent to the APOE locus, is correlated with age of onset differences for Alzheimer's disease (AD). [<http://www.nature.com/tpj/journal/vaop/ncurrent/full/tpj200969a.html>] APOE4 is a well established risk factor for AD and 98% of the time is linked to long forms of the polyT variant. Linked to APOE3 strands, however, are two distinctly different sizes of the polyT repeat, each with distinct evolutionary strand histories within the LD area defined by TOMM40 and APOE and each conferring different disease risk. These data have been replicated, but clinical validation is necessary before use in prediction. A prospective diagnostic validation study designed to follow normal individuals between the age of 62-87 years, combined with a therapeutic clinical trial of a potential "delay of onset" medicine in the high risk group, has been accepted by the FDA Voluntary Exploratory Data Submission review group. An international study involving sites in Russia, England, Switzerland, Australia and the US has initiated identification of normal volunteers so that, once an Investigational New Drug application is in place and a clinical trial with appropriate informed consents can be launched, recruitment into the study will be accelerated. The evolution of the TOMM40 polyT mutations is currently being studied in ethnic-specific populations. The design of the therapeutic trial, the results of the pre-recruitment studies [www.opalstudy.org], and the implications of different risk allele frequencies and evolutionary histories for multinational clinical trials will be presented.

P09.004 Analysis of genes influencing on development of alopecia areata of patients from European part of Russia

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Alopecia areata (AA) is a chronic autoimmune dermatosis with genetic predisposition, primarily affecting the hair and nails. Detection of genes influencing on development of AA is important for understanding the

pathogenesis of the disease.

We have examined the 105 patients living in the European territory of Russia with various forms and stages of AA, including 43 children aged from 3 to 17 years and 62 adult patients aged 19 to 75 years. 70 (66,7%) patients were diagnosed with mild forms of alopecia areata, and 35 (33,3%) patients had severe forms - alopecia totalis (AT) and alopecia universalis (AU).

Subject to clinical features and accompanying diseases we have divided ours patients into groups. The first group included patients with autoimmune polyglandular syndrome type 1 (with endocrinopathy, chronic skin and mucous candidosis, AA), verified by the mutation in AIRE gene R257X in each case. The second group included patients with severe form of AA. Two patients were recognized with the mutation in phospholipase gene LIPH (deletion of the 4th exon). A woman having combination of total form of AA and ichiosis vulgaris was recognized with the mutation 2284del14 in FLG gene.

The third group included patients with various forms of AA. 28 patients were diagnosed with combination of AA and other autoimmune diseases (autoimmune tyroïditis, atop dermatitis, psoriasis, rheumatoid arthritis, vitiligo). We have examined the polymorphism of the TNFA, IL1A, IL1B, IL1RN genes in this group of patients. Some distinctions were revealed.

P09.005 Genetic analysis of *FUS* gene in an Italian cohort of sporadic ALS patients.

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FUS (fused in sarcoma) or *TLS* (translocation in liposarcoma), a DNA/RNA-binding protein, is the cause of ALS6, a dominant autosomal inherited form of Amyotrophic Lateral Sclerosis (ALS). Its main role in the neurodegeneration is highlighted by the presence of cytoplasmic accumulation of its mutant protein form in ALS patients, absent in normal individuals, in SOD1-mutated ALS patients and in sporadic ALS patients positive for TDP-43 aggregates.

The *FUS* gene (chromosome 16p11.2) is encoded by 15 exons and most of the ALS-linked mutations discovered are clustered in the highly conserved extreme C-terminal region.

To further define the frequency and spectrum of *FUS* mutations, we have screened a cohort of 320 Italian patients with sporadic ALS (sALS) coming from South Italy, by analyzing first exons 15 and 14 (mutational hotspots for sALS cases) and then exons 5 and 6. These exons have been analyzed by DHPLC (Denaturing High Performance Liquid Chromatography) screening, except for exon 15 directly sequenced.

We have identified two missense mutations, already described, in 4 patients. The R521G substitution has been observed in 2 patients, as well as the P525L mutation has been found in other 2 cases. In our screening we found also polymorphic variants some of which are novel (3'-UTR variant, c.*41G>A; c.1393+34G>T and c.337-17del[AAAA]). The results of this study combined with other data collections confirm that mutations in *FUS* gene have an important role in pathogenesis of ALS, accounting for at least 1% of the sporadic form of the disease also in our cohort.

P09.006 Grading the credibility of genetic associations in Alzheimer's disease using the Venice criteria: practical considerations following from the Alzgene database

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The epidemiological credibility of genetic associations in Alzheimer's disease (AD) in the 'top list' of the Alzgene database is graded using the so-called Venice criteria. We aimed to evaluate the robustness of these criteria.

Hypothetical results from simulated studies were added to the meta-analyses of these associations that were graded with strong credibility or with moderate or weak credibility due to inconsistency of replication (high between-study heterogeneity (I^2)) or low summary odds ratio (OR < 1.15). Robustness was quantified as the sample size needed to change the grading.

For most associations graded with strong credibility, the grade changed

to weak credibility because of small summary OR after the addition of studies with effects similar to the lowest/highest published OR and sample sizes ranging from 80 to 2000. For half, new studies could introduce large I^2 when their sample sizes were 400 to 1600. These associations ended up with a small summary OR and one became non-significant. Two out of four associations graded with moderate and weak evidence because of $I^2>25\%$, could not become strong evidence because of one outlier in each analysis. Finally, associations with weak credibility due to small OR only became non-significant when new studies with no effect had sample sizes ranging over 3400 to 6600. The Venice criteria are very helpful criteria to grade the credibility of genetic associations, but its practical usefulness may be limited due to outliers and small effects. Further guidance is needed on how to deal with these situations.

P09.007 Overexpression of human amyloid precursor protein causes neurodegeneration and impairment of cognitive functions in transgenic *Drosophila melanogaster*

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Alzheimer disease (AD) is a neurodegenerative disease characterized by accumulation of amyloid deposits, neurofibrillary tangles, neuron death. The main component of amyloid plaques is amyloid- β -protein (A β) - a cleavage product of amyloid precursor protein (APP). According to the main hypothesis primary molecular defects of AD are associated with A β secretion, real mechanisms that lead to neurodegeneration remain unclear.

In our study, transgenic *Drosophila melanogaster* was established as a model to analyze AD-like pathology caused by APP overexpression. *Drosophila* has α - and γ -secretases and does not have activity of β -secretase. Therefore flies do not generate A β . We expected that an elav-GAL4c155 -UAS-driven APP695 and APP695-Swedish overexpression in neural cells of *Drosophila* may induce specific effects of APP independently from A β secretion. We showed a strong deficit of presynaptic proteins in mushroom bodies in flies that expressed full size human APPs. Transgenic lines of *Drosophila* that expressed APP or APP-Swedish exhibited a progressive neurodegeneration with numerous vacuoles in the cortex and neuropil and impairment of cognitive functions. We suggest that impairment of cellular functions of APP and secretion of neurotoxic forms of A β may independently contribute to the synaptic loss in AD.

We used novel peptide mimetics of Apolipoprotein-E, COG112 or COG133 as therapeutic preparation. Selection of apoE-mimetics derived from the receptor-binding region of apoE was based on the ability of these peptides to mimic the functional anti-inflammatory and neuroprotective effects of the intact apoE protein. The development of neurodegeneration and cognitive deficits was corrected by injections of COG112 or COG133.

P09.008 Genetic association of *CCR2* & *CCR5* polymorphisms with Alzheimer's disease

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Chemokines participate in the regulation of immune and inflammatory responses by interacting with their specific receptors on related immune and inflammatory cells such as B-lymphocytes, T-lymphocytes and antigen-presenting cells. Chemokines and their receptors are therefore considered to mediate inflammation and tissue damage in autoimmune disorders. The recent studies have revealed the genotypes of chemokine receptors (CCR) and their related polymorphisms in a number of autoimmune and infectious diseases. We used the polymorphic DNA markers (CCR2-64I) and (CCR5 Δ 32) to study the association of *CCR2* and *CCR5* gene mutations with Late-onset Alzheimer's disease (LOAD) and the relation between clinical features and genotypes in affected individuals. A total of 150 patient samples and 150 healthy controls from west northern Iran (Eastern Azerbaijan) were genotyped for the two polymorphisms by the PCR-RFLP method and genotype frequencies were statistically determined. No significant linkage was determined between *CCR5* Δ 32 and the disease of inter-

est. However the gene *CCR2* was appeared to be significantly linked to the disease, as it could be concluded from statistical analysis.

P09.009 The association of the regulatory region of the PS-2 gene with Alzheimer's disease

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There has been increasing evidence that variants in the promoters and regulatory regions of presenilins (PS1 and PS2) genes may be relevant in the pathogenesis of familial AD (FAD) by altering transcriptional activity. Also, some of those have been reported as risk factors for sporadic AD (SAD) development. Firstly, the PS2 promoter contains multiple putative binding sites for transcription factors and variants in this regulation sequence increases the expression of this gene in vitro experiments. The specific objective of our study was to examine these genetic variants so as to further explore the pathogenesis of FAD and SAD, in an independent series of Southern Italy samples. Clinical data and blood sample were collected from 289 AD patients, after informed consent. The control group consisted of 324 subjects matched for ethnic background, gender and age. Genomic DNA was isolated from blood and a polymorphism, located in the 5' regulatory region of the PS2 (deletion of adenosine at the 24914 site), was assessed by PCR-RFLP (Dde I). There was no statistically significant difference in allelic and genotypic frequency distribution between cases and controls. We also tested whether the different genotypes were associated with clinical features but no significant differences were detected. To conclude, our data suggest that this polymorphism, despite a biological plausibility, do not constitutes a risk factor for AD in our population. However additional intensive association studies with enlarged sample size in different populations are needed, for clarifying the substantial role for PS2 gene promoter polymorphisms in AD.

P09.010 Effect FAD mutations on PS1 mediated cell adhesion and synapse formation

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Growing body of evidence suggests that synaptic loss/dysfunction occurs in the very early stages of Alzheimer's disease (AD). The mechanism(s) by which synaptic dysfunction occurs is not understood. The early-onset familial AD (FAD) caused by mutations in the presenilin 1 (PS1) gene also demonstrates that memory loss and synaptic dysfunction occur in the absence of amyloidogenesis or neurofibrillary degeneration and precede neuronal death. Recent data indicate that PS1 plays an important role in cell-cell adhesion and synapse formation. Therefore, we analyzed effect of FAD mutations on PS1-mediated cell-cell interactions using confocal microscopy and a quantitative assays based on the measuring of number of cellular aggregates and single cells in suspension. L cells stably transfected with inducible GFP-PS1 constructs bearing FAD mutations E318G and G209V showed decreased aggregating activity in comparison with wild-type GFP-PS1. L cells stably transfected with GFP-PS1 constructs, which had a truncated N-terminus, C-terminus or deleted hydrophilic loop of PS1 completely failed to form intercellular contacts and or demonstrated very low aggregating activity. In addition we showed that embryonic neurons transfected with GFP-PS1cDNA bearing FAD mutations displayed a low number of morphological synapses in comparing with neurons expressing wild type GFP-PS1.

P09.011 Neuronal nicotinic acetylcholine receptor alterations as a pathogenic model in sporadic Amyotrophic Lateral Sclerosis

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Sporadic Amyotrophic Lateral Sclerosis (sALS) is a multifactorial neurodegenerative disorder, with unknown aetiology and pathogenesis. We performed a mutational analysis of genes encoding for neuronal nicotinic acetylcholine receptor subunits (nAChRs), considered as candidate genes for their role in motoneurons survival and glutamatergic pathway. Calcium trafficking mediates nAChRs neuromodulating effects, including regulation of cell survival and glutamate release. We sequenced all exons and flanking intronic regions of CHRNA3, CHRNA4 and CHRN β genes in 245 patients (all normal for SOD1) and in 450 healthy control subjects. We found 15 missense variants in patients (6.1%) and 6 variants in controls (1.3%). This difference is statistically significant ($p=0.001$; OR 4.48, 95% CI 1.7 to 11.8). Missense variants were located within the cytoplasmic loop of the receptor, which has a crucial function in modulating channel ionic properties. Tested functionally by mutagenesis, cellular transfection and electrophysiological studies, the observed variants were found to disrupt the receptor properties. Receptors underwent impaired desensitisation and prolonged channel opening, causing a subsequent increase of intracellular Ca^{2+} . We suppose that these mutations could be an important predisposing factor in ALS. Supporting this hypothesis, tobacco smoking is the only proven risk factor for ALS.

Interestingly, three patients with ALS (and none of controls) had a rare missense variant in two distinct subunits of nAChR. These results suggest that epistasis is a major component of the genetic architecture of complex diseases like ALS. This study highlights a possible pathogenic model in sporadic ALS, consisting in a cholinergic dysfunction, that could be pharmacologically targeted.

P09.012 Association study of the interleukin IL17A gene in Caucasian patients with Ankylosing Spondylitis - Preliminary results

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Introduction: IL-17 mediates proinflammatory responses and induces inflammatory cytokine and chemokine production. High expression of IL-17 has been detected in the synovial fluid of spondyloarthropathies (SpA)-affected patients. Furthermore, murine arthritis models deficient in IL17A have shown reduced inflammation.

Objective: Our aim was to investigate IL-17A as a candidate gene for Ankylosing Spondylitis (AS), a member of the group of the seronegative SpA, which characteristic pathological lesion is the inflammation of the enthesis. AS has a strong genetic association with allele B*27 of the human leukocyte antigen (HLA) class I.

Patients and Methods: Genomic DNA samples from 283 HLA-B27 positive individuals were obtained; from those, 139 were diagnosed with AS and the other 144 individuals, although HLA-B27+, were healthy and thereby used as controls. The IL17A promoter, coding regions and intron-exon boundaries were sequenced in the 3130x1 Genetic Analyzer AB.

Results: 5 DNA genetic variants on IL17A gene were identified: -197 G>A (5'UTR region), 27+18 G>A (intron 1), *162 G>A (exon3/3'UTR), *1248 C>T (exon3/3'UTR) and *1252 C>T (exon3/3'UTR). None of these variants was disease specific. Allele frequencies in affected and unaffected individuals were compared using the Fisher's exact test. No statistical significant difference was observed. Conclusion: Our genomic DNA results suggest that IL-17A is not involved in the aetopathogenesis of AS in this population. On the other hand, the number of individuals analysed in this study could be insufficient to evaluate small genetic effects. Further studies should be performed with larger sets of individuals.

P09.013 A common copy number variant (CNV) encompassing two metalloprotease-disintegrin (ADAM) genes is associated with lung function in the ECRHS cohort

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Only few studies have investigated the impact of Copy Number Variants (CNVs) on asthma. We performed a pooling Comparative Genomic Hybridization (CGH) in order to identify CNVs related to asthma, and, in a second stage, we characterized one of the identified CNVs and explored its effect on asthma phenotypes in a European population-based cohort study (ECRHS). Three pools of DNAs from non-atopic asthmatic, atopic asthmatic and control individuals were analyzed by CGH with the Agilent 244K array. A loss on chromosome 8 was detected in the DNA pool from non-atopic asthmatic individuals compared with the DNA pool from the control sample. The CNV was located in a cluster containing several ADAM genes and encompassed the 3' of the ADAM5P gene and the ADAM3A gene. The breakpoints were identical in the individuals analyzed by sequencing. The CNV was genotyped in the HapMap individuals with a FAM labeled PCR based method, and the minor allele frequencies were 8% in YRI, 14% in CHB and 45% in CEU. A SNP (rs11985201) was found to be in high linkage disequilibrium with the CNV ($r^2 > 0.8$), and thus it was used as a proxy to explore the effect of the CNV on asthma phenotypes. In the ECRHS cohort (n=2129), the tag SNP was associated with lung function, mainly with predicted Forced Expiratory Volume in the first second (FEV1%) ($p=0.0067$), and predicted Forced Vital Capacity (FVC%) ($p=0.014$). In summary, a common CNV that encompasses ADAM5P and ADAM3A genes was associated with lung function in the ECRHS cohort.

P09.014 Study of gene-gene interactions for bronchial asthma in Russians from Volga-Ural region of Russia

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Bronchial asthma (BA) is a complex chronic respiratory disease resulting from interactions between multiple genes and environmental factors. In this study we have genotyped 21 polymorphic markers in 15 genes of cytokines (IL4, IL4RA, IL10, TNFA, IL1B, IL1RA), biotransformation of xenobiotics (CYP1A1, CYP2D6, GSTT1, GSTM1, MTHFR, NAT2, CYP2C9, CYP2C19), and a disintegrin and metalloprotease 33 (ADAM33). A total of 295 unrelated participants of Russian ethnic group were recruited, including 172 patients with BA and 123 non-asthmatic individuals from Volga-Ural region of Russia. We found significant associations of IL4(-590C>T), ADAM33(11434C>A) gene polymorphisms with asthma susceptibility. Genotype -590T/T and allele -590T of IL4 gene were associated with an increased risk of atopic asthma (OR=5.04, 95%CI 1.78-14.21; OR=1.88, 95%CI 1.25-2.83, accordingly). Also we found increased frequencies of ADAM33*11434A/A genotype and ADAM33*11434A allele among patients with severe asthma compared with controls (OR=3.14, 95%CI 1.55-6.37; OR=2.02, 95%CI 1.20-3.38, accordingly). The associations remained significant after an adjustment for age. Generalized multifactor dimensionality reduction (GMDR) method was used to analyze gene-gene interactions for bronchial asthma. One two-locus model had a minimum prediction error (testing accuracy 0.59) and a maximum CV consistency (8/10). This model consisted of TNFA(-308G>A) and ADAM33(11434C>A) loci. Significant gene-gene interactions between TNFA (-308G>A), IL10 (-627C>A), IL4 (-590C>T), IL4RA (Ile50Val), IL1RA (VNTR), IL1B (3953C>T) loci were detected for atopic asthma in Russian children

(testing accuracy 0.69; CV consistency (10/10)). For non-atopic asthma, significant interactions were found between IL4 (-590C>T), IL1RA (VNTR), ADAM33 (11434C>A) loci in Russian adult, with CV consistency (10/10) and testing accuracy 0.68.

P09.015 An association of CC16 gene polymorphism with asthma in children

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Background: Clara cell secretory protein 16-kDa (CC16) is an anti-inflammatory protein produced mainly by Clara cells in the distal respiratory lung epithelium. A single-nucleotide polymorphism (SNP) of the CC16 gene (A38G) was previously reported to be associated with asthma.

Objective: To investigate allele and genotype frequencies of the CC16 gene polymorphism in children with asthma.

Patients and methods: Study group: 136 children aged 3 -17 years old including 70 boys and 10 girls with mild asthma and 41 boys, 15 girls with severe asthma. Control group: 127 healthy children (67 boys and 47 girls) aged 4-17 years old. Genetic polymorphism of CC16 was identified by PCR-RFLP (Laing et al., 1998). The data were compared by Chi-square test.

Results: Both allele and genotype distribution of the CC16 gene polymorphism were similar in asthma patients and in controls being line with previously reported date. No difference was found when comparing CC16 gene polymorphism between groups: mild asthma - severe asthma, severe asthma - control, mild asthma - control. However we observed a significant difference in prevalence of 38A allele in asthmatic girls compared to girls from control group (88% vs 58%, $\chi^2=7.528$, $p=0.023$, OR=1.5 95% CI=1.17 - 1.95). Additionally, of 38A allele frequencies was significantly higher in asthmatic girls compared to asthmatic boys (88% vs 64%, $\chi^2=8.414$, $p=0.015$, OR=1.5 95% CI=1.23 - 1.89).

Conclusion: We proposed that CC16 gene polymorphism A38G might be predisposing factor of asthma development in girls.

P09.016 ABCG1 transporter in reverse cholesterol transport and atherosclerosis development.

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Transport of excess cholesterol by high density lipoproteins (HDL) from macrophages in the periphery back to the liver, called reverse cholesterol transport (RCT), plays an important protective role in the development of atherosclerosis. Macrophage ATP binding cassette transporter G1 (ABCG1) mediates cholesterol efflux to HDL. However its role in human atherosclerosis development is undiscovered.

The aim of this study was to investigate the influence of ABCG1 gene expression variations on atherosclerosis development. Human peripheral blood monocytes were obtained from 25 volunteers: 15 patients with angiographically proved atherosclerosis and 10 healthy blood donors. Monocytes were cultured with macrophage colony-stimulating factor (M-CSF) for 24 hours. ABCG1 mRNA levels in monocytes derived macrophages were measured using real time PCR and normalized to beta-actin. ABCG1 gene expression level in macrophages of patients with atherosclerosis doesn't significantly differ from the same value for healthy control: average macrophage ABCG1 mRNA levels for patients and for healthy individuals are 0.87 ± 0.66 and 1.26 ± 0.55 , respectively, ($p=0.076$). Simultaneously there is positive correlation between macrophage ABCG1 mRNA level and HDL cholesterol ($R>0.65$, $p<0.02$). These results suggest that ABCG1 transporter regulates HDL metabolism and cholesterol content and thereby protects against atherosclerosis.

The study was supported by Russian Foundation for Basic Research (grant 06-04-49609).

P09.017 Investigating the relationship between premature atherosclerosis and Heme oxygenase 1, Heme oxygenase 2 and Kalirin gene in myocardial infarction

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Atherosclerosis is the most important cause of Cardio-vascular disease and early and sudden onset which its occurrence is related to various factors. Oxidant and oxidative stress are among the environmental factors which cause endothelial vessels hurt. Heme oxygenase 1 and 2 and Kalirin genes decrease oxidative stress and restrict hurt of endothelial vessels. The aim of this study is investigating the SNPs of promotor region of Heme oxygenase 1 and Heme oxygenase 2 genes and study of kalirin genes in patients and control group.

Method: Blood sampling was done on 150 patients and 100 normal persons.

Results: investigating of promotor part of Heme oxygenase 1, shows that SNPs No.rs1805173, rs13057211 and rs3074372 with ($P < 0.001$) were meaningful, but SNPs No.rs13057211 and rs3074372 weren't meaningful.

By investigating the Heme oxygenase 2 in the exon 5, substitutions of A to G in K89E codone (AAG to GAS) were observed as meaningful ($P < 0.01$).

By investigating of SNPs (rs578477) and (rs 9289231) in kalirin gene show that significant and meaningful correlation with premature atherosclerosis ($P < 0.01$).

Discussion: The investigation of the results shows that there is correlation in Iranian population to affecting premature atherosclerosis with SNPs of promotor part of Heme oxygenase 1 the substitution of A to G Heme oxygenase 2 and above mentioned SNPs (in result section) of kalirin genes show that there is a meaningful correlation to increasing susceptibility to premature atherosclerosis among Iranian population.

P09.018 Detection of epistatic effects of FCER1A variants on eczema risk

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Allergic diseases are mostly driven by high levels of total and allergen-specific IgE levels. The high-affinity receptor for IgE is composed of three subunits *FCER1A*, *FCER1B*, *FCER1G* and represents the central receptor of IgE-induced reactions. A genome-wide association analysis revealed associations between functional *FCER1A* variants and total serum IgE levels. Linkage and association of *FCER1B* variants with IgE and atopic diseases had been reported by previous studies. The *FCER1G* subunit has not yet been investigated with regard to atopy. Filaggrin (*FLG*) is the strongest known risk gene for eczema, in particular the allergic subtype of eczema.

We investigated the association of *FCER1A*, *FCER1B* and *FCER1G* variants with IgE in a large population-based cohort. We further tested for epistatic effects using the model-based multifactor dimensionality reduction method (MBMDR) and investigated a potential interaction between *FLG* and *FCER1A* variants in a eczema case cohort and population controls.

We found association of three strongly correlated *FCER1A* variants with total and specific IgE levels as well as allergic sensitization. No associations could be detected for *FCER1B* and *FCER1G*. After adjusting for *FLG* effects, a significant epistatic effect of the *FCER1A* variants rs10489854 and rs2511211 on eczema risk revealed.

We conclude that *FCER1A* variants by themselves and in combination have an impact of IgE levels and act synergistically to influence eczema risk.

P09.019 Association analysis of two common filaggrin mutations (2282del4 and R501X) and atopic diseases in Volga-Ural region of Russia.

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Atopic diseases, such as atopic dermatitis (AD), bronchial asthma (BA) and allergic rhinitis (AR), are common multifactorial diseases. Recent studies have suggested that epidermal barrier defect may play a crucial role in the development of atopic diseases. Filaggrin is a key protein involved in skin barrier function. Mutations in the *FLG* gene are an important predisposing factor for AD, BA with AD and systemic allergies. Two mutations (2282del4 and R501X) in the *FLG* gene are common in people of European origin.

The purpose of this study was analysis of the role of *FLG* mutations in the susceptibility to AD, BA and AR in Russians, Tatars and Bashkirs from Volga-Ural region of Russia. The atopy group consisted of 519 BA patients, 284 AD patients and 265 AR patients; the control group included of 266 individuals. The carrier frequency of 2282del4 mutation was 10% in AD Russian patients, 14.2% in Tatars and 21.4% in Bashkirs. In AR group, the frequency of 2282del4 was 7.54% in Russians, 14.28% in Tatars and Bashkirs. This mutation was present in 15.78% asthma Russian patients with AD, 22.22% in Tatars and 25% in Bashkirs. The frequency of 2282del4 was significantly lower in control group (2.36% in Russians, 1.76% in Tatars and 5.8% in Bashkirs). The R501X mutation was rarely found in our cohort: only 1.76% atopy patients carried R501X mutation.

The data of this study revealed a strong association of the *FLG* mutation 2282del4 with AD, BA with AD and AR in Russians, Tatars and Bashkirs.

P09.020 A large public health effect of a common variant on chromosome 11q13 (rs7927894) on eczema, asthma, and hay fever

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In a genome-wide association study for eczema, a common variant on chromosome 11q13.5 (rs7927894) has been identified as a susceptibility locus in four study populations from Central Europe. We aimed to determine the effect of this risk variant on various allergic diseases on the population level. More than 7,400 individuals from a large English birth cohort born in 1991 and 1992, were genotyped for rs7927894. Association analyses were performed for eczema, asthma, hay fever, allergic sensitization, and combinations of these phenotypes. The effect of the risk variant on eczema was restricted to individuals with atopic eczema (OR, 1.31; 95% CI, 1.07-1.60). In contrast, no association of rs7927894 with non-atopic eczema was observed. Moreover, we detected association with concomitant asthma (OR, 1.50; 95% CI, 1.14-1.97) and hay fever (OR, 1.54; 95% CI, 1.15-2.06). The population attributable risk fraction for atopic eczema was estimated to be 23.8%. The rs7927894 risk allele is a common variant that confers a moderate risk for atopic disease, but carries a large public health effect on atopic eczema and allergic airways disease. Furthermore, the association with atopic eczema as well as concomitant asthma and hay fever points to a key role in the atopic march.

P09.021 Identification and characterization of copy number variations in 50 children with autism

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Autism is a neurodevelopmental disorder that manifests in the first three years of life. The group of pervasive developmental disorders (PDDs), also termed autism spectrum disorders (ASDs), includes the prototypical autism as well as PDD-not otherwise specified (PDD),

Rett syndrome and Asperger's disorder. Autism is characterized by difficulties in managing social interaction and communication, and a limited, repetitive and stereotyped patterns of reactions and behavior. ASDs are etiologically heterogeneous, and only 10% of the cases are due to known medical conditions (chromosomal imbalances, genetic disorders like Fragile X, Rett syndrome or Tuberous sclerosis). The emerge of new high-resolution screening methods allows for detection of submicroscopic genomic aberrations. One of these methods; microarray-based comparative genomic hybridization (aCGH) offers the possibility of detecting copy number variations (CNVs, deletions or duplications) associated with autism.

We present the results of applying aCGH for identification of causal CNVs in 50 children diagnosed with autism. Genomic DNA was extracted from buccal cells and aCGH was performed using 44k and 105k human genome CGH oligo microarrays (Agilent Technologies Inc.). Causal CNVs were identified in 9 (18%) of the patients. Two patients had large aberrations (unbalanced translocation between chromosome 8 and chromosome 12, idic(15)). Five patients had smaller CNVs in or close to genes previously associated with autism, and two patients had CNVs not previously reported. Further analyses are needed to determine the significance of these aberrations. Our data demonstrates the utility of aCGH as a powerful diagnostic tool for detection of genomic imbalances in ASD.

P09.022 Common variants in cadherin 10 gene show association with autism spectrum disorders in Finnish population

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Autism spectrum disorders (ASDs) are severe neurodevelopmental disorders that are highly heritable, but phenotypic and genetic heterogeneity have made it challenging to identify predisposing variants across populations. Although 10-20% of ASD cases are known to be caused by rare high-penetrance mutations, cytogenetic abnormalities, and *de novo* DNA copy number variation, genome-wide association studies (GWAS) with large sample sizes have also revealed common genetic variants associated with ASDs. Recently, several candidate genes have been shown in independent studies to be associated with ASDs in different populations. To test for association between ASDs and genetic variants in these genes in the Finnish founder population, we have genotyped 79 single nucleotide polymorphisms (SNPs) in eight candidate genes in families with autism (n=142) or Asperger syndrome (AS; n=121) and performed association analyses. In the autism study sample, the most significant association was observed with an intronic SNP in *CDH10*, rs1505874 ($P=0.000066$). In the AS study sample, the most significant association was also observed in *CDH10*, but with intronic rs6867043 ($P=0.000774$). In a recent GWAS (Wang *et al.* 2009), signals with a strong genome-wide significance were observed in six SNPs located between *CDH10* and *CDH9*. Results from our study support the role of *CDH10* in ASDs using an independent sample. To our knowledge, this is the first replicated association between ASDs and *CDH10* reported. *CDH10* is involved in neuronal cell adhesion and in the development of synaptic complexes so our results further support the hypothesis that neuronal cell-adhesion are implicated in the etiology of ASDs.

P09.023 Association of CTLA-4 and PTPN22 genes polymorphisms with autoimmune thyroiditis in Tatar population

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Autoimmune thyroiditis (AITD) is a common disorder characterized by the presence of thyroid-specific antibodies against a variety of antigens of thyroid tissue, such as thyroid peroxidase (TPO) and thyroglobulin (TG). Current evidence suggests that susceptibility to AITD is likely to be influenced by the combined effects of several genes and their interactions with environmental factors. We genotyped CTLA-4 variants: -1661 G/A, -318 T/C, +49 G/A and variant 1858 T/C of PTPN22 gene in 161 Tatar AITD cases and 137 age/sex matched healthy individuals. Genotypes were determined by the PCR-RFLP method as described earlier. Data was analyzed using Chi-square test and 95% confidential interval (CI). The frequency of CTLA-4 -1661 G allele and genotype

A/G and +49 G/A G allele and genotype GG carriers was significantly higher in patients than in controls ($P=0.04$, OR 1.84, 95% CI 2.31-1.4; $P=0.001$, OR 2.0 95% CI 1.62-2.31 respectively). We showed that the carriers of A/G, T/C and G/G genotypes of -1661 A/G, -318 T/C and +49 G/A polymorphisms have an increased risk of genetic predisposition to the AITD in Tatar women (OR 7.87, 95% CI 2.03-3.25). Also strong association was observed between the increased level of antibodies to TPO (> 1000 ME/I) and GG genotype of +49 G/A polymorphism (OR 1.3, 95% CI 1.5-4.1); and antibodies to TG (> 100 ME/I) and genotypes A/G and G/G of CTLA-4 -1661 A/G and +49 G/A polymorphisms (OR 1.56, 95% CI 2.25-3.6; OR 1.12, 95% CI 1.9-2.75 respectively).

P09.024 Strategies to identify new mutations in patients with Bardet-Biedl syndrome.

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Bardet-Biedl syndrome (BBS, MIM 209900) is a rare multiorganic disorder which a variable phenotype that includes retinal dystrophy, polydactyl, mental delay, obesity and also reproductive tract and renal abnormalities. The combination of the late onset of some of the features of BBS, such as renal disease and loss of vision, and the existence of other genetic syndromes with similar cardinal manifestations can lead to confusion amongst clinicians and the possibility of misdiagnosis.

Until now 14 genes (BBS1-BBS14) have been involved in 70% of the families, indicating that additional mutations in known BBS genes and new BBS genes remain to be identified.

We utilized a BBS genotyping chip by Asper Ophthalmics to perform first screening of the samples. The latest version of the chip contains 107 mutations from BBS1-7, BBS9, BBS10 and BBS12. In the case of BBS8, only SNPs have been included. In five cases a single heterozygous mutation was found in BBS1 (p.M390R), BBS2 (p.Y89C) and BBS10 (C91fsX). The entire open reading frame of the gene containing those mutations was directly sequenced in order to detect an additional novel causative mutation within that gene. Novel mutations were detected in BBS1 and BBS10 families, two missense mutation and two deletions, respectively. Nevertheless, no additional mutations were detected in the BBS2 coding sequence.

The combination of the BBS genotyping chip and sequencing of genes where a heterozygous mutation was detected, seem to be a good strategy to find new BBS mutations.

P09.025 Case - control association study of candidate genes and genome - wide association study in Bulgarian patients with bipolar affective disorder

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Bipolar affective disorder is a severe psychiatric illness characterized by episodes of mania and depression. Although the etiology is not clear, epidemiological studies suggest it is a result of an interaction of genetic and environmental factors. Genetic studies performed so far, however, failed to identify the definite genetic variants associated with the disease. Our study encloses two approaches. The candidate gene approach was conducted prior to and as a part of the genome - wide

association scan. In the first screening of the candidate gene approach 191 SNPs were genotyped in 94 Bulgarian patients and 184 Bulgarian healthy individuals. SNPs that revealed P value less than 0.05 were genotyped in the second screening using an additional independent set of samples consisting of 78 cases and 372 controls. One variant, rs1800883, in the HTR5A gene revealed a significant level of P value ($P=0.000097$; odds ratio=1.80 (95%CI, 1.27-2.54); corrected $P=0.017$) suggesting the plausible role of this gene in the pathogenesis of bipolar disorder in Bulgarian population. In the second stage, we conducted a genome-wide association scan followed by a replication study of the top 100 SNPs. The GWAS was performed on 188 BAD patients and 376 control subjects genotyped on the Illumina 550 platform. The replication study was conducted on 122 BAD cases and 328 controls. Although our GWAS did not reveal any strong association and none of the top 100 SNPs reached the Bonferroni-corrected P value in the replication study, the plausible involvement of some variants cannot be entirely discarded.

P09.026 MTHFR 677TT and PON1 55MM homozygotes are associated with early occurrence of coronary artery disease but not aortoiliac occlusive disease

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The methylenetetrahydrofolate reductase (MTHFR) 677C>T, 1298A>C and paraoxonase 1 (PON1) -108C>T, L55M, Q192R polymorphisms, which influence homocysteine metabolism were studied for the possible specificity of involvement in early development of the atherosclerotic diseases: coronary artery disease (CAD) and aortoiliac occlusive disease (AIOD).

Altogether 619 men, age below 60 years, were recruited: 300 subjects with CAD, 144 men with advanced stage of AIOD (scheduled for elective surgery) and 175 control men without symptoms of vascular diseases. The severity of CAD was examined by coronary angiography and expressed by the number of affected vessels (one-vessel, two-vessel or three-vessel disease). The differences in allele, haplotype and genotype distributions were studied.

The frequency of MTHFR 677TT homozygotes in CAD (10.3%) was higher than in AIOD (4.2%; OR=2.7; $p=0.03$). The frequency of this genotype in CAD subjects with three-vessel disease was 4.9-fold higher than in AIOD ($p=0.001$) and 4.1-fold higher than in CAD subjects with one-vessel disease (4.8%; $p=0.01$). The frequency of PON1 55MM homozygotes in CAD patients was twice of that in the AIOD+control group ($p=0.01$), but no associations were seen between PON1 polymorphisms and advancement of CAD.

In conclusion, the MTHFR 677TT genotype seems to be the specific risk factor for both early occurrence and advancement of CAD, whereas the PON1 55MM increases risk only of CAD development before 60. Presented analysis shows that the studied genetic background in CAD differs from that in AIOD. Supported by grants MNiL N40208131/2499 and 2P05C03828.

P09.027 Genetic factors for congenital anomalies of the kidneys and urinary tract (CAKUT): results from the AGORA project

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Congenital Anomalies of the Kidneys and Urinary Tract (CAKUT) occur frequently in man and comprise the most common cause of end-stage renal failure in children. Disorders belonging to the spectrum of these anomalies include renal agenesis, multicystic kidney dysplasia, and duplex collecting system. Not much is known about the origin of

CAKUT. Alterations in genes expressed during nephrogenesis are considered important, with the final phenotypic outcome depending on additional modifying genetic and environmental factors. The aim of this study is to identify genetic factors involved in CAKUT aetiology. From the AGORA biobank of the Radboud University Nijmegen Medical Centre over 700 CAKUT case-parent triads and 10 families with multiple affected members were included in this study, comprising a unique and well-documented CAKUT cohort. Mutation analysis of CAKUT candidate genes is ongoing by sequencing coding regions and intron-exon boundaries. Several interesting genetic variants were identified and subsequently functionally tested *in vitro*. In addition, linkage analysis was performed in a large CAKUT family with 10 affected individuals. This demonstrated suggestive linkage at a 9 Mb locus on chromosome 1. Deep sequencing of this linkage interval is performed to identify the genetic variants involved. In an additional 4 families, genome-wide exome sequencing was performed. Identification of genetic factors involved in CAKUT facilitates the understanding of its pathogenesis and enables the design of genetic diagnostic screening tests with a view to early detection and recurrence risk estimations for CAKUT.

P09.028 Association between eNOS gene polymorphisms and cannabinoid dependence

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Endothelial nitric oxide (eNOS) regulates the production of vasodilatory nitric oxide (NO). Recent studies demonstrated that association between eNOS polymorphism and/or NO production and behavioral changes. The aim of this study whether polymorphisms in eNOS gene are associated with cannabinoid users in Turkish population.

Our study, 94 cases cannabinoid users and 100 age-and sex-matched healthy controls were tested for two polymorphisms which were Glu298Asp (G894T) and intron 4 VNTR polymorphisms in eNOS gene. Genotyping were performed by PCR and/or RFLP.

The distribution of AA, AB and BB genotypes for intron 4 VNTR polymorphism was 59%, 28% and 13% in cases compared with 72%, 25% and 3% in the controls ($p=0.008$). The allele frequency of A and B was 0.729, 0.271 in cases compared with 0.845, 0.155 in the controls ($p=0.006$). Also, the distribution of GG, GT and TT genotypes for Glu298Asp polymorphism was 46%, 40% and 14% in cases compared with 65%, 35% and 0% in the controls ($p=0.03$). The allele frequency of G and T was 0.660, 0.340 in cases compared with 0.825, 0.175 in the controls ($p=0.0001$). The observed genotype counts was deviated significantly from those expected according to the Hardy-Weinberg Equilibrium ($p=0.008$ for intron 4 VNTR, $p=0.03$ for Glu298Asp).

This study is the first to search eNOS gene polymorphisms in cannabinoid users. We conclude that two eNOS gene polymorphisms were associated with either distribution of genotypes and the allele frequencies in cannabinoid users. Identification of genetic events in addiction may provide clues about etiology and therapeutic targets.

P09.029 Novel Variations in Cardiac Troponin T Gene associated with Cardiomyopathy - An Indian Study

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Cardiomyopathy, a group of primary cardiac muscle disorders including dilated (DCM) and hypertrophic (HCM) cardiomyopathy, is one of the major causes of heart failure and sudden death. The disease is phenotypically heterogeneous. Some individuals remain asymptomatic throughout life, others develop progressive symptoms, or experience SCD. The genetic basis for disease are mutations identified in sarcomeric genes.

Cardiac troponin T (cTnT) mutations in particular predispose the affected to SCD. Troponin T (TnT), together with troponin C (TnC), troponin I (TnI), and tropomyosin, act as regulatory proteins of the sarcomere. TnT is the most important regulatory protein of the sarcomere, and exists in many alternatively spliced isoforms. Expression studies have shown that residues 70-170 forming a part of TnT tail are crucial

for TnT binding to tropomyosin, tropomyosin binding to actin, and stabilization of the complex.

The cTnT gene was screened in 309 cardiomyopathy patients (162 HCM, 147 DCM) and 176 controls. The study identified 2 missense mutations, 5 silent mutations, 14 intronic variations. Of the 21 variations obtained, 11 are novel variations. We identified a novel R144W missense mutation in exon 10 in DCM, predicted to inhibit the force generation by blocking calcium activation of thin filament. The mutation also results in loss of recognition site of several restriction enzymes and thus can be used as diagnostic test. In silico analysis of other variations revealed that they are likely to alter the splicing efficiency or effect the binding capacity of snRNPs, which will be discussed.

P09.030 Investigation of causative mutation in four generation family with celiac disease using next generation sequencing and genome wide genotyping array

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Celiac disease (CD), caused by a strong immune response to gluten, is the most common food intolerance in Western populations. CD is strongly associated to human leukocyte a antigen (HLA) DQ2/DQ8 gene which explains some 35% of the heritable risk. A genome-wide association study for celiac disease identified 26 other loci which together explain only another 5% of the heritable risk. To identify additional CD genes we performed whole-genome linkage in a four-generation Dutch family. We identified a dominantly inherited linkage region at chromosome 9p21-13 and a second one on chromosome 6q25 from the model-free analysis. We hypothesize that these regions may contain a high risk mutation that plays a causal role in the disease development in this family.

In order to investigate copy number variation (CNVs) and find new candidate loci which could have been missed in the linkage analysis due to the large spacing between microsatellites, we genotyped 32 out of 46 family members (18 affected, 14 unaffected including 4 spouses) using a high-density SNP CytoChip array. We performed CNV analysis using two independent algorithms and found a duplication on chromosome 9q34.3 which segregates through all four generations and is present in 8 affected and 1 unaffected family members, but is not present in the spouses. Although we did not find any interesting genes in this region, our duplication might contain regulatory sequences affecting the expression of others genes.

We are currently applying a high-throughput exome sequencing approach to identify point mutations and/or small deletions in linkage regions.

P09.031 Allele-specific gene expression (ASGE) of novel celiac disease associated SNPs

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Eleven SNPs within eight genomic regions displayed strong genetic association with celiac disease (CD) in GWAS and follow-up studies: rs2816316 (1q31, *RGS1*), rs13015714 and rs917997 (2q11-12, *IL18RAP*), rs6441961 (3p21, *CCR1/CCR3/CCR2*), rs17810546 and rs9811792 (3q25-26, *IL12A/SCHIP1*), rs1464510 (3q28, *LPP*), rs13119723 and rs6822844 (4q27, *KIAA1109/Tenr/IL2/IL21*), rs1738074 (6q25, *TAGAP*) and rs3184504 (12q24, *SH2B3*). Of these, only rs3184504 locates within the coding region of a gene.

Intronic and intergenic SNPs have to a great extent, and surprisingly, showed to be subject to allele-specific gene expression (ASGE) and are hence consistent with the existence of numerous unannotated gene transcripts in the human genome. The source of ASGE is assigned to *cis*-acting factors such as polymorphisms in the flanking regions of the gene.

The aim of our study is to explore *cis*-effects by performing correlation of gene expression and SNP genotypes, as well as to investigate ASGE of SNPs found in transcripts from CD relevant tissue.

So far we have studied expression of five SNPs. Two SNPs, rs1464510 (early intron of *LPP*) and rs1738074 (UTR of *TAGAP*), showed to be expressed and all heterozygous individuals will next be subjected to ASGE analysis for these SNPs. For the untranscribed SNPs (e.g. rs2816316, rs17810546 and rs9811792) we will perform real-time

PCR expression of neighboring genes and correlate gene expression with SNP genotypes.

P09.032 Association of a complex CNV region on 8p21.2 with child and adult obesity in the French population

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Obesity is a growing public health concern exhibiting a high level of heritability, much of which is unaccounted for. We have sought to investigate the contribution of copy number variants (CNVs) to the genetic susceptibility to child and adult obesity.

We have applied our novel CNV prediction algorithm cnvHap to Illumina GWAS data from child and adult obesity case-control cohorts from Northern France. CNVs were identified within a region on 8p21.2 encompassing a SNP showing marginal association with obesity in the combined child and adult obesity dataset ($p=5.68\times 10^{-4}$). Further investigation of this CNV region (CNVR) revealed it to encompass two variable number tandem repeats (VNTRs) and a 3,975bp deletion. The 3,975bp deletion and surrounding VNTRs were subsequently genotyped using a combination of PCR and fragment analysis in the Northern French child obesity cohort of 688 cases and 592 controls and adult obesity cohort of 693 cases and 776 controls. This revealed significant individual associations of both VNTRs and the deletion with obesity ($p<0.05$). Since these variants are each only weakly significant, we consider it likely that the genotyped VNTRs and deletion are tagging an as yet unidentified causal variant in this region.

Detailed investigation of the 8p21.2 region has highlighted the complex genomic architecture of many CNVRs, and emphasises the benefits of the application of an approach combining bioinformatics and molecular biology to CNV discovery and genotyping.

P09.033 Systematic evaluation of the clinical significance of inherited and *de novo* Copy Number Variations in families with Autism Spectrum Disorders

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Array-based studies have detected a wealth of copy number variations (CNVs) in patients with autism spectrum disorders (ASD). Since some CNVs also occur in healthy individuals their contributions to the patient's phenotype remain largely unclear. We systematically evaluated the clinical significance of *de novo* and inherited CNVs containing genes being transcribed in the brain, by taking into account the Social Responsiveness Scale (SRS) scores of patients and both parents. Thus, CNVs were grouped into four categories. First, *de novo* CNVs in families with both parents having normal SRS scores, and second, CNVs inherited from a carrier parent with an elevated SRS score. These are the most likely to be causally related to ASD. The genes in these two categories participate in pathways probably associated with ASD, such as contactin-based cell communication (*CNTN5*, *CNTN6*), phosphoinositol signaling (*PIK3CA*), and regulation of neurotransmitter levels (*KCNMB4*, *TPH2*). The *de novo* CNVs found in patients with at least one parent scoring higher than 1 SD above the mean on the SRS, and the CNVs inherited from an unaffected parent, while the other parent has an elevated SRS score, are likely to exert a minor contribution to the phenotype. Our analysis also indicates that in 6 out of 48 ASD patients two or more genetic etiologies may be involved. This study extends the scope of genome wide CNV profiling beyond *de novo* CNVs in sporadic patients, and may constitute a first step toward uncovering the missing heritability in genome wide screening studies of complex psychiatric disorders.

P09.034 Genome wide analysis of coeliac disease patients with atypical HLA risk genotypes

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Coeliac disease is a multifactorial disorder caused by the ingestion of gluten in genetically susceptible individuals. The genes encoding the HLA DQ2 and DQ8 heterodimers are the strongest genetic risk factors for the disease, roughly 90% of patients carry DQ2 in cis (DQ2.5) or trans (DQ2.2 and DQ7) and 5-10% have DQ8. These HLA variants are also common in the general population, implicating additional genetic factors in disease susceptibility.

A recent GWA study of coeliac disease revealed up to 40 risk loci for CD, and included 647 samples from the Finnish population. 24 Finnish patients were found to not carry the most common DQ2.5 or DQ8 risk haplotypes. We performed a more detailed HLA typing and found out that 15 of these carried only half of the DQ2 risk molecules, either DQ2.2 (n=15) or DQ7 haplotype (n=1) alone. 1 patient was totally negative and 7 had the trans genotype (DQ2.2 + DQ7).

We performed an HLA stratified genome wide association analysis and found an association on chromosome 1 ($p<10^{-5}$) in the group of patients and controls without the DQ2.5 or DQ8 haplotypes. Furthermore, we have performed detailed phenotypic analysis of these individuals to determine possible atypical coeliac disease in individuals with atypical HLA risk variants.

P09.035 A TWO-WAY APPROACH FOR DETECTING LOCI CONFERRING SUSCEPTIBILITY TO COLORECTAL CANCER

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Complex diseases, such as colorectal cancer (CRC) are thought to follow a polygenic model of inheritance in which many low-penetrance alleles in several susceptibility loci interact to determine which individuals finally develop the condition. Following this premise, the determination of such susceptibility loci has mainly focused on two directions: the whole-genome approaches, and the candidate gene selection, in which the screening involves a series of genes that are particularly thought to be involved in the evolution of the disease. We have followed the latter approach by two different means: the selection of candidate genes derived from the fifteen mouse *Susceptibility to colorectal cancer* (Scc) regions, and the determination of the variation in the genes belonging to two pathways related to tumorigenesis: Wnt and BMP4. In total, 190 SNPs were chosen from 41 genes to be genotyped in stage one in 515 cases and 515 controls belonging to the EPICOLON I project. The three top-associated SNPs were further replicated in an independent series of 933 cases and 955 controls (EPICOLON II). Although none of the SNPs were finally proved to be associated with the disease, we believe the *CYR61* gene and its surrounding region should be further investigated in order to clarify any possible association with CRC.

P09.036 Transcriptome analysis in Complex Regional Pain Syndrome: new insights into inflammatory and neuroplasticity signaling pathways

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Background: The molecular basis of Complex Regional Pain Syndrome (CRPS) is not known and its diagnosis remains essentially clinical. A constellation of clinical manifestations are present in CRPS accounting for central and peripheral alterations (e.g., pain, edema, inflammation, hyperalgesia, allodynia) in which immunological and neurotransmitter system imbalance have been indicated to play a pivotal role. Gene expression studies using peripheral blood samples have been broadly used for the study and identification of metabolic candidate pathways as well as immunological diseases. We propose that such a study is also worthwhile in CRPS with its mixture of central and peripheral hallmarks in which circulating immune cells may be taken as a surrogate of neuronal systems hallmarks.

Methods: The study was performed on well characterized CRPS cases and matched controls and involved *in vitro* stimulation of whole peripheral blood mononuclear cells (PBMCs) before and after stimulation with inflammatory triggers. After RNA isolation, amplification and fluorescent labelling, analysis was carried out using Illumina's BeadChip 8v3 protocol. Differentially expressed were analyzed using Ingenuity Pathway Analysis (IPA v7).

Results: Differentially expressed genes (DEGs) between CRPS cases and matched-controls were significantly observed only after *in vitro* exposure to inflammatory triggers. Similar basal expression patterns between CRPS cases and matched controls were detected.

Conclusions: Preliminary results showed evidence of subtle but significant changes among molecules involved in inflammatory pathways (only) after *in vitro* exposure to inflammatory triggers. The study opens a new window for the identification of common inflammatory molecules that may be involved in CRPS development.

P09.037 SMURF1 is involved in cardiac development and congenital heart defects

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Congenital heart defects (CHDs) are characterized by a large spectrum of structural anomalies of the heart. Occurring in about 1 % of newborns CHDs are the most common developmental anomaly. ArrayCGH screening identified a 486 kbp *de novo* duplication of 7q22.1 in a patient with coarctation of the aorta. The region contains three genes: *TMEM130*, *TRRAP* and *SMURF1*. Bioinformatics prioritization identified *SMURF1*, a regulator of TGF-beta signalling, as a strong candidate gene for heart development and CHD. *SMURF1* interacts with 6 known heart proteins and is highly expressed in human embryonic and fetal heart. Immunohistochemical analysis of human fetal hearts revealed that the *SMURF1* protein shows strong, spatially and temporally restricted expression during heart development. During differentiation of the P19. CL6 mouse stem cell line, which can differentiate into beating cardiomyocytes, *SMURF1* expression is up regulated.

The functional effect on cardiac development for *SMURF1* was investigated using the zebrafish as a model. Together, our findings suggest a function for *SMURF1* in heart development and CHD.

P09.038 COPACETIC, a genome-wide association study on chronic obstructive pulmonary disease (COPD).

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Chronic obstructive pulmonary disease (COPD) is characterized by persistent, progressive airway obstruction. COPD is a complex disease which develops due to an interplay of environmental factors like

tobacco smoking and genetic factors which are still largely unknown. The aim of our study is to identify novel genetic factors for COPD using a genome-wide association study (GWAS). Study participants were recruited from the Nelson study, a CT-based lung cancer screening trial in heavy-smokers of Dutch/Belgium descent (>20 pack years). DNA samples were genotyped using Illumina Human610-Quad arrays. COPD was defined both as obstruction (FEV1/FVC10% of lung volume with a density 70% and FEV1%pred >90. To gain statistical power we also used blood bank controls with unknown smoking history which were genotyped using Illumina Human670-Quad arrays.

Association tests performed on 1030 obstruction cases and 1799 controls resulted in 81 SNPs with p value $< 1 \times 10^{-4}$. The top 384 SNPs for obstruction were selected for a currently ongoing replication in five independent (population-based) cohorts comprising a total of 3,603 obstruction cases and 7,207 controls.

Our GWAS study on COPD is unique since it includes both CT scan data and lung function for each participant, as well as a detailed smoking history. Therefore we expect to identify loci associated with COPD in heavy-smokers and possibly also loci that distinguish between the different COPD phenotypes emphysema and airway obstruction.

P09.039 β -defensin gene cluster copy number analysis in Celiac Disease

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BACKGROUND: Celiac disease (CD) is a complex, immune-mediated disorder of the intestinal mucosa with a strong genetic component. The nature of CD pathogenesis remains unclear, but both innate and adaptive immune responses are involved in the development of the disease. Human β -defensins are highly inducible, anti-microbial peptides which form part of the innate immunity. Genomic copy number of β -defensin gene cluster is polymorphic (with most individuals possessing 4 copies) and has been associated with autoimmune or inflammatory disorders including psoriasis or Crohn's disease.

AIM: Our aim was to determine whether β -defensin cluster copy number variants are associated with CD.

PATIENTS AND METHODS: *DEFB4*, *DEFB103* and *DEFB104* gene copy number was performed by real-time PCR using specific Taqman probes and primers in 376 CD patients and 376 control individuals. Gene-specific experiments were performed in sextuplicate (duplicate for each defensin gene) using 4 ng of genomic DNA. Raw data were normalized to endogenous *Rnase P* gene and were analyzed using Copy Caller software assuming 4 to be the most common copy number. Comparisons between groups were performed using χ^2 .

RESULTS: The distribution of samples in to >4, 4 and <4 copy number bins was 25.9%, 42.5% and 31.6% for patients and 33.5%, 37.0% and 29.5% for controls, respectively.

COMMENTARY: β -defensin gene cluster copy number distribution differed between celiac and non-celiac groups, showing a trend to smaller proportion of high copy numbers in CD-patients.

P09.040 Genetic variations of MGP gene promoter and the risk of coronary artery disease

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Coronary Artery Disease (CAD) is a major cause of death worldwide. Positive family history among patients points to the significance of genetic elements in the risk of CAD. Two single nucleotide polymorphisms (SNPs) at -7 & -138 positions on Matrix- Gla Protein (MGP) gene have been suggested to play a role in susceptibility to CAD. Such a role is possibly due to the effect of these SNPs on the level of gene expression.

This study aims to investigate the association of these SNPs with CAD in Iranian population and their possible effects on MGP gene promoter

activity in cultured cells.

To achieve these goals, a population-based genetic association study is being conducted on appropriate groups of patients and controls. Analysis of the preliminary data obtained from genotyping shows an excess of A allele at position -7 and T allele at position -138 among patients. The observed differences are not significant at this point, but genotyping of proposed sample size has not been completed yet. On the other hand, 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 revealed significant differences in GFP expression by different haplotypes of promoter (Student's T-test: $p < 0.0005$). The maximum and minimum activities are seen by promoters containing A-T and G-C haplotypes respectively.

P09.041 Study of rs10757274 and rs2383206 polymorphisms on chromosome 9 as CAD genetic risk factor in sample of Iranian population

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Coronary artery disease (CAD) is the major cause of death worldwide. genome-wide association studies have reported several SNPs on chromosome 9p21.3 associated with increasing CAD risk. Association of rs10757274 and rs2383206 were studied with CAD in 111 cases and 100 controls from Iranian population. Pairs of primers were designed to amplify DNA fragments containing SNPs by Mismatch PCR-RFLP technique. The frequencies of genotypes AA, AG and GG For rs10757274 in cases respectively were 11.7%, 36.9% and 51.4% and in controls were 12%, 54% and 34%. These frequencies for rs2383206 in cases were 9%, 36% and 55% and in controls were 9%, 53% and 36% respectively. Statistical analysis showed significant associations between rs10757274, ($P=0.029$) and rs2383206, ($P=0.036$) polymorphisms in Iranian population. Association analysis of GG/GG haplotype for rs10757274, rs2383206 presumed a significant increase in the risk of CAD. Frequency of GG/GG haplotype was 43% in cases ($P=0.014$, $X^2=6.058$). The risk of CAD with 90% confidence intervals (CI) were estimated between 34 to 52 percent.

P09.042 Association of CETP gene polymorphisms with coronary heart disease in Tatar population

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Cholesteryl ester transfer protein (CETP) is one of the HDL modifying proteins that have both pro- and anti-atherogenic properties. Cholesteryl ester transfer protein (CETP) gene polymorphism is known to be associated with changes in lipid profiles. Primary hyperlipidemia is considered to be a major risk factor for atherosclerosis and coronary heart disease (CHD).

Herein, we investigated the association of several polymorphisms in the CETP gene (Val421Ile, -971G/A and -629C/A) with genetic susceptibility to CHD in Tatar population.

The study included 125 patients with coronary heart disease and 135 health individuals. Genotypes were determined by the PCR-RFLP method as described earlier. Data was analyzed using Chi-square test and 95% confidential interval (CI).

Plasma CETP activity was significantly ($P < 0.001$) higher in primary combined hyperlipidaemic individuals than in controls. Plasma HDL-C was higher in both groups, in the B2B2 genotype than in the B1B1 and B1B2 genotypes, whereas the serum TG concentrations and CETP activity were lower in B2B2 genotype compared with other genotypes (B1B1 and B1B2). The genotype and allelic frequencies for this polymorphism differed significantly between hyperlipidaemic and nonlipidaemic individuals ($P < 0.05$). In both groups, CETP *Taq 1B* polymorphism (presence of B2 allele) correlated significantly with HDL-cholesterol (HDL-C) ($r=0.201$ and $r=0.452$ in control and patient groups respectively) and CETP activity ($r= -0.123$ for controls and $r= -0.192$ for patients).

P09.043 Platelet glycoprotein receptor Polymorphism as a risk factor for coronary thrombosis - A Hospital based study.

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The *GPⅡb/Ⅲa* complex is an integrin class of adhesion molecule receptors expressed abundantly on platelet membrane with an important role in platelet physiology. The receptor mediates the interaction of activated platelets with ligands, including fibrinogen, von Willebrand factor, vitronectin, and fibronectin. Polymorphisms in platelet membrane glycoprotein genes can alter their antigenicity, regulate their expression levels, and modulate their functional properties. It is also known to be involved in the pathogenesis of acute coronary syndromes. Hence in this study, we analyzed the *PⅠ^{A2}* polymorphism at position 1565 in exon 2 of the glycoprotein *Ⅲa* gene by allele specific restriction digestion PCR to identify its importance in the pathogenesis of myocardial infarction (MI). The study cohort included 97 patients with a diagnosis of MI and 112 normal individuals. *A¹A¹* genotype was seen in 57/97 (58.7%), *A¹A²* in 20/97 (20.61%) and *A²A²* in 20/97 (20.61%) patients with MI; while in control group, 94/112 (83.92%) had *A¹A¹* genotype, 14/112 (12.5%) *A¹A²* and 4/112 (3.57%) carried *A²A²*. The prevalence of *PⅠ^{A2}* was 2.4 times higher among the case patients than among the controls (41.23% Vs 16.07%, $P = <0.0001$). This study suggests a strong association between the *PⅠ^{A2}* polymorphism of the glycoprotein *Ⅲa* gene and acute coronary thrombosis. Hence, genotyping will enable identifying the risk of an individual to develop MI and also take prophylactic measures.

P09.044 Variety of inflammatory bowel disease genotypes in Russian patients.

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Inflammatory bowel disease includes two clinical subtypes: Crohn's disease (CD) and ulcerative colitis (UC).

Association of several SNPs of *NOD2*, *DLG5*, *TLR4*, *OCTN1*, *OCTN2*, *IL1*, *IL10* and *TNF α* genes with CD and UC was shown.

We investigated these SNPs in our samples. Samples from 108 unrelated patients with CD, 107 unrelated patients with UC and 179 population controls from Russia were examined. DNA was screened for such SNPs as R702W, G908R, c.ins3020C of *NOD2* gene, R30Q of *DLG5*, D299G of *TLR4*, L503F of *OCTN1*, c.-207G>C of *OCTN2*, VNTR of *IL1*, c.-1082G>A of *IL10* and c.-308G>C of *TNF α* gene.

The allele frequency is shown in table.

| Allele fr-cy SNPs | N | CD | UC |
|-------------------|------|---|---|
| R702W | 0.03 | 0.04 ($p>0.673$) | 0.03 ($p>0.99$) |
| G908R | 0.01 | 0.01 ($p>0.99$) | 0.02 ($p>0.75$) |
| c.ins3020C | 0.04 | 0.18 ($p<0.001$) | 0.03 ($p>0.83$) |
| R30Q | 0.07 | 0.18 ($p<0.001$) | 0.06 ($p>0.739$) |
| D299G | 0.13 | 0.07 ($p<0.056$) | 0.06 ($p<0.007$) |
| L503F | 0.29 | 0.38 ($p<0.017$) | 0.38 ($p<0.021$) |
| c.-207G>C | 0.34 | 0.37 ($p>0.59$) | 0.36 ($p>0.718$) |
| VNTR | 0.27 | 0.31 ($p>0.449$) | 0.27 ($p>0.847$) |
| c.-1082G>A | 0 | 0 ($p<0.0001$) | 0 ($p<0.0001$) |
| c.-308G>C | 0.12 | 0.12 ($p>0.896$) | 0.1 ($p>0.594$) |

Association with investigated SNPs with CD was found for c.ins3020C of *NOD2*, R30Q of *DLG5*, L503F of *OCTN1*, c.-1082G>A of *IL10*; and with UC for D299G of *TLR4*, L503F of *OCTN1*, c.-1082G>A of *IL10*.

On the basis of given results it is possible to assume that *NOD2*, *DLG5*, *OCTN1* and *IL10* genes play a part in development of Crohn's disease. And *TLR4*, *OCTN1* and *IL10* genes play a part in development of ulcerative colitis.

P09.045 Single clone intron deletion detected on BAC Array led to the diagnosis Duchennes Muscular Dystrophy in boy with mental retardation and behavioural disturbances

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Background:

Duchenne muscular dystrophy (DMD) is a X-linked recessive disorder caused by mutations in the DMD gene, which encodes the protein dystrophin. One third of DMD cases are caused by de novo mutations in the DMD gene. The absence of dystrophin causes progressive, non-reversible muscle cell degeneration. DMD can be associated by mild mental retardation and neuropsychiatric disorders such as autism and ADHD.

Case study:

A five year old boy was referred to us by Children's Psychiatric Hospital. The indication for genetic analysis was mental retardation. The boy also showed general developmental delay and attention deficit.

Chromosome analysis and Southern Blot analysis for Fragile X syndrome gave normal results. BAC Array CGH (Cytochip 2.0) revealed a single clone deletion on Xp21.2 in intron 2 of the DMD gene. To confirm the BAC Array results, we made a MLPA analysis, which surprisingly revealed a duplication of exon 3-7 of the DMD gene, which will often give a DMD phenotype. Finally, we did a follow-up study in an oligonucleotide array-CGH analysis (Agilent 180K), where the complex DNA copy number changes were not clearly resolved.

The referring physician later confirmed that the boy also had muscle weakness. Elevated level of creatine kinase confirmed the diagnosis.

P09.046 Association of the variable number of tandem repeats (VNTR) polymorphism in 3'-untranslated region (3'UTR) of the dopamine transporter gene DAT1/SLC6A3 with alcoholism in Russian population of Siberian region

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Some studies have reported that the A9 allele of the VNTR of the gene which encodes the dopamine transporter (DAT1/SLC6A3) is associated with alcohol dependence and alcoholism withdrawal symptoms. We investigated VNTR polymorphism of DAT1 gene in three groups: alcoholic men, men and women with alcoholic, viral and mixed etiology cirrhosis and apparently healthy men control group. The genotype frequencies obeyed the Hardy-Weinberg equilibrium in all groups. The association of DAT1 VNTR with alcoholism was found ($p=0.059$ in genotype level and $p=0.025$ in allele level). In patients with liver cirrhosis statistically significant genotype frequencies difference between men and women was revealed ($p=0.038$). In this group, and in subgroups of men and women no correlation DAT1 variability with age, cirrhosis, alcoholism and virus carrying period, alcohol doze, cirrhosis severity, cirrhosis etiology, stage of alcoholism, smoking were found. There were no differences (in allele frequency) between control and total group of cirrhosis patients as well as between alcoholic and mixed etiology cirrhosis men and women. Comparison of 10/10 genotype frequencies against other genotypes revealed near significant difference between alcoholic and mixed etiology cirrhosis ($p=0.059$). At the same time, patients with cirrhosis only with alcoholic etiology significantly differed from control ($p=0.033$) due to decreased frequency of 10/10 genotype. Thus, DAT1 VNTR polymorphism is associated with the alcoholism without cirrhosis and alcoholic cirrhosis but not with cirrhosis mixed (alcohol and virus) etiology in Russian population of Siberian region. This work was supported by the Russian Foundation for Basic Research (project no. 09-04-99083-r_ofi).

P09.047 Variations in the 7q32-q34 region containing CHRM2, PTN and DGK α are associated with dyslexia in Finnish and German populations.

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We present a genetic study on dyslexia using samples from two independent populations. Dyslexia is a common disability in children with adequate educational opportunities, intelligence, neurological and sensory functions. The *DYX1C1*, *ROBO1*, *DCDC2* and *KIAA0319* genes have so far been the most studied but clearly there are more genetic factors accounting for the complex phenotype of reduced cognitive activities of reading and writing. We present here results from a fine-mapping approach in Finnish and German dyslectic cohorts. A previously reported region of suggestive linkage (NPL=2.8) with dyslexia was restricted with 15 additional markers followed by genotyping of 143 SNPs spanning the region. To increase the power and study the effect of stratification for severity of the phenotype we then genotyped 50 SNPs across 1 Mb in an extended sample set of Finnish families with dyslexia and 1050 individuals (of which 251 affected) from the German population as a replication sample. Several haplotypes show significant association with dyslexia between rs2350780 and rs273933 on chromosome 7q32-q34. This region harbours the *CHRM2*, *PTN* and *DGKI* genes. Several haplotypes overlapping in both populations were included in intron 1 of *DGKI* (diacylglycerol kinase, iota). All three genes in this region are expressed in brain. Pleiotrophin (PTN) has a neurite extension activity and PTN-knockout mice display lower threshold for long-term potentiation in hippocampus, thus suggesting a role in learning and memory. We hereby confirm and refine a dyslexia susceptibility locus harbouring three functionally interesting candidate genes on chromosome 7q34 in two independent European populations.

P09.048 Screening for genes involved in dyslexia using MLPA technique in Brazilian individuals

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Dyslexia is defined as a disorder or learning disorder in the area of reading, writing and spelling, not as a result of poor literacy, lack of attention, motivation, socio-economic status or low intelligence, but as a hereditary condition. Complex diseases such as dyslexia are usually caused by several susceptibility genes that lead to a similar phenotype. Currently, there are 4 prominent genes associated with dyslexia: *DYX1C1*, *KIA00319*, *DCDC2* and *ROBO1*. The method MLPA (Multiplex Ligation-dependent probe amplification) was used in this study to evaluate deletions/duplications in genes associated with dyslexia. The P150 Dyslexia probe mix contains probes for most of the *DCDC2* exons. In addition, several probes for the *KAAG1*, *KIAA0319* and *NRSN1* (VMP) genes that are nearby *DCDC2*, as well as probes for the *ROBO1* and *ROBO2* genes on chromosome 3p12 are present. In this work 14 dyslexics of Brazilian origin, were analyzed by MLPA technique. The DNA fragments generated in reaction using the P150 kit were separated by capillary electrophoresis in DNA sequencer ABI PRISM 310. Our results indicate that none of the patients showed changes in copy number of probes and therefore no duplication or deletion was observed so far in the regions detected by probes on the kit. Finding genes that contribute to the phenotype of complex diseases represents one of the biggest challenges. The identification of these genes, the elucidation of their functions, create windows before whom she can view the complex molecular mechanisms, allowing for real possibilities of new treatments.

P09.049 Autosomal Dominant Dyslexia Pedigree Maps to 7q21

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Reading disability (RDG) has been shown to be comorbid with Rolandic Epilepsy (RE), with RE probands having a 6-fold increase in risk for RDG and their unaffected siblings a 3-4-fold increased risk. We therefore set out to map a locus for RDG in RE families, using multipoint linkage analysis on 25 2-3 generational pedigrees ascertained in the United States. The max HLOD obtained was 3.10 on 7q21 at D7S660, under a dominant mode of inheritance, 60% penetrance. Of these 25 families, 16 provided positive LOD scores, with the majority of the linkage evidence coming from 8 French-Canadian families. One particular French-Canadian pedigree with 20 individuals (14 genotyped), 11 of which are RDG affected, provided a multipoint LOD score of 2.10 at D7S660 under a dominant mode of inheritance with 99% penetrance, with D7S660 providing the maximum multipoint LOD score genome-wide. This pedigree appears to be inheriting this trait in an autosomal dominant fashion. D7S660 sits in a gene-sparse region, however, there is one gene very close to this microsatellite. We are in the process of re-sequencing genes at this locus in the French-Canadian pedigree.

P09.050 A novel mutation in the *Dj1* gene found in an early onset Parkinson's disease patient

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Parkinson's disease (PD) is the second most common neurodegenerative disease. PD is considered as a complex disease, and both genetic and environmental factors contribute to its etiology. Although PD is generally a disease of the elderly, early onset cases constitute a small subgroup of the patients. Among the genes known to be associated with PD, *PRKN*, *Dj1* and *Pink1* are most relevant to early-onset Parkinson disease. *PRKN* encodes an E3 ubiquitin ligase, *Dj1* codes a protein that apparently plays a neuroprotective role and is involved in the cellular response to oxidative stress, and *Pink1* codes a serine/threonine protein kinase that localizes to mitochondria. In the study being reported, we screened *Dj1* and *Pink1* by direct sequencing in 10 Iranian PD patients with age of onset of less than 30 years. Early onset patients were chosen in whom mutations in *PRKN* had been ruled out by previous mutation screening of this gene. No mutation in the eight exons of *Pink1* was found. One homozygous variation, c.G319C in exon 5 of *Dj1* was observed in one patient. This novel variation causes the substitution of alanine at position 107 by proline (A107P). This non-conservative substitution occurs at a turn connecting β 7 and α 5 secondary structures in the protein. Further analysis is needed to confirm the pathogenic effect of this mutation.

P09.051 Mutation screening of *LTBP2* in Ectopia Lentis patients

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Ectopia Lentis (EL) is a pathologic condition in which the lens in the eye becomes displaced from its normal position. EL sometimes manifests as an isolated condition termed simple EL, but more frequently as one of the symptoms of a syndrome. Marfan syndrome (MFS) and Weill-Marchesani syndrome (WMS) are diseases often associated with EL. To date, Fibrillin (FBN1) is the only gene linked to EL. The protein coded by FBN1 is a major microfibrillar component of the extracellular matrix (ECM). Latent TGF β Binding protein 2 (LTBP2) is also a microfibrillar component of the extracellular matrix with high structural similarity to Fibrillin, suggesting it too may be a causative gene for ocular disorders involving the ECM. Recently LTBP2 was identified as a Primary Congenital Glaucoma (PCG) causing gene. Several PCG patients harboring LTBP2 mutations were observed to be also affected with EL. The association of LTBP2 with Fibrillin in the extracellular matrix, and the observation of EL in some PCG patients harboring LTBP2

mutations prompted a further investigation of possible role of LTBP2 in promoting EL. We therefore screened all 36 exons of LTBP2 in 7 simple EL, 6 MFS, and 2 WMS patients, all of whom exhibited lens displacement. In addition to several known polymorphisms, a truncating null mutation in exon 7 of the gene was observed in an MFS patient.

P09.052 Two Novel mutations in SCN1A gene in Iranian Epileptic Patient

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Epilepsy as a common chronic neurological disorder is characterized by recurrent unprovoked seizures. Febrile seizures are the most common type of epilepsy in infants and children. Our aim was the molecular analysis of SCN1A gene in affected Iranian patients with GEFS+ and Dravet syndrome diagnosed clinically to explain genotype-phenotype correlation and exact classification.

Materials and Method: The 34 unrelated Iranian families with epilepsy were selected and screened for SCN1A mutations by MLPA, ARMS, and PCR-RFLP confirmed by direct Sequencing.

Results: MLPA analysis showed normal pattern, but the direct sequencing revealed that generally 20 of 34 (0.588) probands have a common reported SNPs (p.A1067G; rs2298771), with allelic frequency as 0.706 / 0.294 in patients and 0.515 / 0.485 in control group respectively for A/G. No significant differences between two groups were observed. Moreover four novel allelic variants as missense substitutions included two new sequence variation (p.F412 I, p.Y1274N) and two previously reported mutations (p.R101G, p.S103G) which were detected in 4 of 34 probands, but not in control groups and other healthy normal family members.

Conclusions: It seems that the clinical diagnosis could nearly establish the classification, but mutation screening helps clinicians to confirm their data. We found mutation in four probands and confirmed the net diagnosis. Our data suggest that the clinical sign variations could be also explained considering the role of modifier genes such as mitochondrial mutations or other genes responsible for drug metabolism pathways including multiple drug resistance family genes (ABCB1) or MTHFR.

P09.053 distribution of the CyP1B1 and CYP2F1 gene variants in three ethnic groups of Bashkortostan and case-control study with COPD.

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The aim of this study was to appear interethnic variation interethnic variation in frequency distribution patterns of CYP1B1 and CYP2F1 genes polymorphic markers and association analysis with chronic obstructive pulmonary disease The polymorphic markers Leu432Val (CYP1B1) and c.14_15insC (CYP2F1) were studied at chronic obstructive pulmonary disease patients (COPD) (N=306) and cases of healthy individuals (N=467) (Russian, Tatar and Bashkir), residents of Bashkortostan.

It was shown that the CYP2F1 gene genotype frequency distribution patterns differed between three ethnic groups ($\chi^2=21.29$, df=4, $p=0.0001$), because of high frequency of c.14_15insC / c.14_15insC genotype in Tatars, whereas in Bashkirs and Russians it was 1.28% and 0.53%, respectively. On the other hand, high frequency (39.74%) of wild type/ c.14_15insC genotype was appeared in Bashkirs and only 29.95% in Russians and 20.21% in Tatars.

Association analysis of CYP2F1 gene insertion variant with COPD have shown high frequency of wild type (86.7%) allele in patients with very severe stage and manifestation of COPD after 55 years ($\chi^2=4.584$, df=1, $P=0.032$; OR=1.799 95%CI 1.048-3.099). It was shown that allele and genotype frequency distribution of Leu432Val CYP1B1 gene not differed between Russians, Tatars and Bashkir ethnic groups ($\chi^2=8.433$, df=4, $p=0.077$ and $\chi^2=3.474$, df=2, $p=0.176$). We did not find any association of CYP1B1 gene with COPD.

P09.054 Silent c.3306C>T (p.L1102L) mutation in ABCB4 gene causes exon skipping in patient with Primary Sclerosing Cholangitis

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Primary Sclerosing Cholangitis (PSC) is an idiopathic chronic cholestatic disorder described as multifactorial disease in human. However, Abcb4 knockout mice (Abcb4 -/-) associated with absence of biliary phosphatidylcholine evolves in a progressive cholangiopathy leading to a severe form of PSC while in human the absence of the orthologous ABCB4 protein causes progressive familial intrahepatic cholestasis type 3 frequently characterized by rapid evolution to end stage liver disease.

We investigated the ABCB4 gene by sequencing of the 27 coding exons and by qualitative cDNA analysis in two subjects with PSC disease.

Sequencing analysis identified in one 13 year old boy the silent nucleotide change c.3306C>T (p.L1102L) in exon 26. Searches for putative Esonic and Intronic Splicing Enhancers (ESEs and ISEs, respectively) by ESE-Finder program has predicted that this silent mutation causes disruption of SRp40 motif identified in this locus. Qualitative mRNA analysis of ABCB4 gene has subsequently showed an heterozygous exon 26-skipping event that causes a deletion of 76 amino acid located in the Nucleotide binding domain-COOH terminal, but retaining the correct open reading frame.

This study identifies the first ABCB4 silent mutation that causes the skipping of exon 26 producing a deleterious allele in a child with PSC disease. We conclude that also silent mutations in the ABCB4 gene need further characterization at mRNA level in order to exclude the potential pathogenic role in subjects with suspected ABCB4 deficiency.

P09.055 Filaggrin mutations and their impact on stratum corneum lipid levels and other skin barrier parameters in patients with eczema

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The discovery of loss-of-function mutations in the filaggrin gene as cause of the monogenic keratinisation disorder ichthyosis vulgaris and strong risk factors for eczema was a major breakthrough in complex disease genetics. Alterations in ceramide levels, changes in skin pH and increased trans-epidermal water loss (TEWL) are characteristic in eczema patients. However, until now no studies have evaluated a potential impact of FLG deficiency on stratum corneum lipid levels and other skin barrier parameters.

A cohort of 49 German individuals genotyped for the two common FLG mutations (R501X, 2282del4) had stratum corneum samples taken for lipid analysis by high performance thin layer chromatography (HPTLC), and TEWL, erythema, skin hydration and pH were measured. In 27 individuals an additional 24-hour irritation patch test with sodium lauryl sulphate (SLS) was performed. For analysis, both the eczema group and the control group were stratified by FLG mutation status (FLGmut/ FLGwt).

Eczema patients with FLG mutations had significantly higher erythema compared to patients without FLG mutations. FLGmut individuals had significantly higher skin pH values than FLGwt individuals. In the FLGmut eczema group significantly lower levels of ceramide 4 and significantly higher levels of ceramide 7 were observed compared to both healthy control groups. However, ceramide 7 levels also significantly

differed between FLGwt eczema and FLGwt controls, as did ceramide 1 levels. No significant differences were observed for ceramide 2, 3, 5 and 6.

Our results approved previous findings of altered ceramide levels in eczema, which however appear to show no clear relationship to FLG mutations.

P09.056 Search for genetic variants associated with low folate status

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Results of genome-wide association studies indicate that private mutations may play an important role in the pathogenesis of complex diseases. Since low folate status has been implicated in birth defects, cancer and cardiovascular disease we searched in candidate genes for allelic variants associated with low folate status. From a historical cohort of 591 controls and 296 patients with coronaryographically confirmed coronary artery disease (Janošková et al, Mol Genet Metab 79, 2003:167-75) we have selected 22 controls and 12 patients with both plasma and erythrocyte folates below the 10th percentile of the distribution. In these subjects we have resequenced the coding region of exons and the adjacent 50-300bp intronic regions in five genes involved in folate metabolism and transport. Sequence analysis of the *FOLR1*, *FOLR2*, *FOLR3*, *MTHFR* and *RFC* genes in all subjects revealed 5, 1, 2, 3, and 2 novel variants, respectively, that have not been previously deposited in the SNP database. Although some of these variants were synonymous mutations, in 15 of 34 subjects with low folate status we have identified either missense mutations or putative splicing/termination codon mutations. Functional testing of these candidate variants and their frequency analysis in the entire cohort are now being carried out. This study indicates that private mutations in *FOLR1*, *FOLR3*, *MTHFR* and *RFC* genes may be associated with low folate status and may thus contribute to the susceptibility for several complex diseases. This work has been supported by the grant No. NS10036-4/2008 from the Ministry of Health of the Czech Republic.

P09.057 The obesity-associated SNPs in intron 1 of the *FTO* gene affect primary transcript levels.

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As shown by genome-wide association studies, single nucleotide polymorphisms (SNPs) in intron 1 of the *FTO* gene are associated with the body mass index and type 2 diabetes, although the functional significance of these SNPs has remained unclear. Previous studies have not revealed any effect of the *FTO* genotype on total mRNA levels of the *FTO* gene or the neighbouring *RPGRIP1L* gene. Such studies, however, are confounded by the fact that unrelated individuals unavoidably differ in genetic background, environmental exposure and life events so that subtle effects cannot be detected. This problem can be circumvented by determining the ratio of allelic transcript levels in heterozygous individuals, where each allele serves as an internal control for the other. To measure allelic transcript ratios, we developed highly quantitative fluorescence-tagged single nucleotide primer extension assays. For *FTO*, we used unspliced heterogeneous nuclear RNA and SNP rs9939609. Allelic expression ratios of the neighbouring *RPGRIP1L* gene were investigated in individuals who were heterozygous for the *RPGRIP1L* SNP rs4784319 and heterozygous or homozygous for the *FTO* SNP rs9939609. In each of five different blood samples tested, the *FTO* transcripts containing the A (risk) allele of rs9939609 were more abundant than those with the T allele (mean 1.38; 95% confidence interval 1.31 to 1.44). Similar results were obtained in fibroblasts and adipocytes. We also observed skewed allelic expression of the *RPGRIP1L* gene, but skewing was independent of the *FTO* genotype. Our data suggest that increased expression of *FTO* leads to increased body weight.

P09.058 Gene-gene interaction of LKB1-AMPK-TORC2 pathway components exert an important influence on the occurrence of T2D in Japanese.

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The LKB1-AMPK-TORC2 signaling pathway controls glucose homeostasis in the liver, and mediates therapeutic effects of insulin sensitizing antidiabetic agents. Given the putative biological interactions among LKB1, AMPK a2-subunit and TORC2 proteins, to examine whether gene-gene interaction in the three genes (STK11, PRKAA2, CRTC2) encoding component of this signaling pathway increase susceptibility to type 2 diabetes, we tested for statistical interactions among SNPs in the three genes with respect to T2D susceptibility. To limit multiple testing, we used ht-SNP data sets to represent each gene or locus, and the pairwise gene-gene (locus-locus) interactions were analyzed by GAIA (Genetic Association Interaction Analysis), a recently developed statistical method for evaluating gene-gene interactions. we observed significant and specific interactions between SNPs in STK11 and CRTC2 genes, while no interactions were found with either between the STK11 and CRTC2 SNPs, at least three pairs of SNPs showed a statistically significant interaction with respect to T2D risk (rs3829686-rs2072704, rs6510599-rs2072704 and rs741765-rs2072704, with the most significant pair (rs6510599-rs2072704) reaching a p-value of 0.0010 (Permutation-p = 0.019, under an "additive only" model). Interestingly, individual SNPs that were found to interact did not necessarily show significant single-locus associations in our previous study, except for rs741765. Our findings suggest that gene-gene (SNP-SNP) interaction studies between STK11 and CRTC2 influencing susceptibility to type 2 diabetes in Japanese.

P09.059 An Italian Network of isolated populations for genetic dissection of complex traits

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The use of isolated populations to reduce disease heterogeneity of complex disorders is a modern and valid instrument to study genetic basis of complex diseases. In order to better study multifactorial traits we have created a network (INGI) that join different isolated villages in Italy. We choose the populations in order to represent Italian variability and differences between nord and south. At this moment there are about 1700 people from Val Borbera (nord-west), 1400 from Friuli Venezia Giulia (nord east) and 700 from Carlantino (south).

In the isolates we have performed a fully clinical evaluation including anamnesis, anthropometrical measurements, three blood pressure estimates, a clinical chemistry, evaluation bone densitometry, ECG. We have also created a DNA and sera banks. Some specialistic examinations were performed: cardiological (ECG, echocardiogram), odontiatric (orthopantomography), neurological, psychiatric examination and bone densitometry and audiometry evaluations. We have also collected information on dietary habits.

Moreover we have collected genealogical data using church and municipality registers.

All samples were genotyped using ILLUMINA 370K SNPs-Array and inferred using HapMap 2M data. We are currently performing inferences of all the populations using data derived from 1000 genomes consortium. Thanks to the high number of phenotypes collected we are involved in different consortia for the identification of genetic factors for common traits (heart traits like QT and PR intervals, hypertension, white and red blood cells, platelets and menarche). We are currently performing association analysis for POF(premature ovarian failure), bone density, cardiac frequency, etc. but we are open to all possible collaborations.

P09.060 IRAK3 gene variants associate with atopic asthma: a replication study in the Spanish population

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Asthma is a chronic inflammatory lung disease that is affected by both genetic and environmental factors. *IRAK3* has recently emerged as a new candidate gene for susceptibility as a result of positional cloning of early onset persistent asthma in Sardinian families, with two SNPs further replicated in a case-control sample of atopic persistent asthma from Italy. Here we aimed to replicate the association of *IRAK3* common variants with susceptibility to asthma.

Using re-sequencing data of 23 kilobases of non-repetitive gene regions from 32 unrelated healthy subjects, we selected a set of 15 tagging SNPs (tagSNPs), forcing the inclusion of predicted functional variants and previously associated SNPs. Genotyping was conducted by means of SNaPshot® Multiplex System in 611 asthmatic cases and 1407 population-based controls from the Genetics Of Asthma study (GOA) in the Spanish population.

Variants of *IRAK3* showed no association with susceptibility to asthma, rhinitis, atopic dermatitis or total IgE. However, multiple SNPs distributed across the gene associated with early onset asthma ($0.009 \leq p\text{-value} \leq 0.043$; $q\text{-value} < 0.10$) and atopic asthma ($0.016 \leq p\text{-value} \leq 0.050$; $q\text{-value} < 0.05$), replicating the two previously associated SNPs from the Italian study. Finally, a stratified analysis across Italian and Spanish samples further supported the strength of the associations ($\min p = 1 \times 10^{-5}$ in a joint analysis over > 2500 individuals).

Taken together, our results confirm that variants of *IRAK3* gene might play an important role in early onset and atopic asthma pathogenesis. Supported by FUNCIS 23/07 and grants from the Spanish Ministry of Science and Innovation PI081383 and EMER07/001 to CF.

P09.061 Genome wide association study identifies three loci associated with psoriatic arthritis

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Psoriatic arthritis (PsA) is an inflammatory joint disease distinct from other chronic arthritides and frequently accompanied by psoriasis vulgaris and seronegativity for rheumatoid factor. In order to identify susceptibility genes, we performed a SNP array based genome wide association study in 609 German patients and 990 control individuals.

Analysis of 1,585,307 confidently imputed SNPs revealed three loci achieving genome-wide significance ($p < 5 \times 10^{-8}$) that were replicated in independent study groups of 1761 patients and 3727 control individuals of European origin. We confirmed *HLA-C* ($p = 2.63 \times 10^{-23}$) and *IL12B* ($p = 5.60 \times 10^{-13}$) as PsA susceptibility genes and observed several independent signals within the HLA region. The most associated *IL12B* variant, located 72kb upstream of the gene, was independent of previously reported psoriasis-associated *IL12B*-SNPs. Association to several intragenic variants of the *TRAF3IP2* gene ($p = 5.48 \times 10^{-9}$) including the missense-variant R74W (rs13190932) was detected implicating this component of the IL-17 receptor signalling pathway¹ as a PsA susceptibility locus. Our study implicates new susceptibility variants at known susceptibility loci and in addition identifies a novel pathway for PsA.

P09.062 The role and frequency of glutathione S-transferase P1 polymorphism in Iranian patients affected with reflux esophagitis

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Background: Reflux esophagitis is a common complication of the gasto-esophageal reflux disease. Glutathione S-transferases (GSTs) have important role in the protection of cells from the products of oxidative stress. *GSTP1**B allele has a correlation with susceptibility to several diseases. In this case-control study, the role and frequency of *GSTP1* polymorphism was evaluated in Iranian patients with erosive reflux esophagitis.

Methods: Seventy patients with reflux esophagitis and seventy-five normal individuals were enrolled in this study. The grade of esophagitis was determined via endoscopy. DNA was extracted from venous blood of each subject using the salting out method. *GSTP1* genetic polymorphisms were detected using the PCR-RFLP method.

Results: There was a significant difference between the frequency of non-variant (*A/*A) and variant *GSTP1* genotypes (*A/*B, *B/*B) in both patient and normal groups ($P = 0.006$). Also in the patient group, the grade B of esophagitis was significantly associated with variant *GSTP1* genotype ($P = 0.028$). The rate of throat pain symptom was higher in the no-variant group ($P < 0.036$).

Conclusion: The *GSTP1**B allele frequency in Iranian patients with reflux esophagitis and normal control groups is similar to Orientals. In addition, *GSTP1* polymorphism is correlated with a higher grade of esophagitis.

P09.063 The CA haplotype (-174C, -597A) of the IL-6 gene and systemic sclerosis susceptibility

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Systemic Sclerosis (SSc) is a complex multiorgan disease. The key steps in its pathogenesis include dysregulation of the immune system and early endothelial damage followed by the fibroblast activation. The interleukin-6 (IL-6) gene is implicated in all of these pathogenic pathways.

Objectives: to investigate the influence of the -174 G/C, -597 G/A SNP and their haplotypes on the susceptibility of SSc.

Materials and Methods: We assessed 118 European Caucasians subjects (67 SSc patients -86.5%F/13.5%M and 51 healthy controls -83%F/17%M) for -174G/C and -597 G/A SNP by PCR-RFLP method (Hsp 92II, Fok I as specific restriction enzymes). We used for all the statistical analyses the software package PLINK v1.07. The haplotype-association tests were based on the expected number of haplotypes each individual has and was studied by the chi-squared test.

Results: The genotype frequencies (Wigginton test) respected Hardy-Weinberg equilibrium for the controls but not for the SSc group. The association analysis (Fischer test) showed no difference in the distribution of allele frequencies regarding both SNP positions between SSc patients and controls. Four common haplotypes were identified. Among them, the CA haplotype (-174C, -597A) was present in 13.7% SSc patients and 25.8% controls. We found a negative association ($p=0.02$) between the CA haplotype and SSc suggesting that this haplotype exert a protective effect against disease susceptibility.

Conclusions: This study shows no association of the IL-6 -174 (G/C) and -597 (G/A) polymorphisms alone with SSc, but the CA haplotype (-174C, -597A) could confer a disease resistance in investigated population.

P09.064 Contribution of genetic factors to the variance in the frequency and severity of hereditary angioedema episodes

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Hereditary angioedema (HAE) is a rare autosomal disorder caused by the deficiency of C1-inhibitor (C1INH) and characterized by recurrent episodes of angioedema. Frequency and localization of the edemic bouts are quite different sometimes even between members of the same family carrying identical C1INH mutation. Beside various triggering factors the different genetic background may contribute to the variation of symptoms. We aimed to evaluate the association of five polymorphisms of different genes related to the pathogenesis of the disease with the frequency and localization of edemic episodes. Moreover, correlation of C1INH concentrate usage with the occurrence of these polymorphisms was also analyzed.

One hundred and twenty-nine Caucasian HAE patients were enrolled in the study. The genotype of a 9 bp insertion/deletion polymorphism of the bradykinin B2 receptor gene, three single nucleotide polymorphisms of exon 1 (alleles A/B/C/D) within the gene of mannose-binding lectin and the copy numbers of complement component C4 genes were determined applying PCR based methods. The potential influence of different C1INH mutations on disease severity was also evaluated.

We found that the analyzed polymorphisms are associated with the frequency and localization of attacks. Our data indicate that the studied variations are independent predictors of HAE attack frequency and C1INH concentrate usage irrespectively of the carried C1INH mutation.

Characterization of genetic factors influencing severity of edemic attacks may help in the near future to find the best therapeutic intervention for hereditary angioedema patients with different symptoms.

P09.065 HHEX-IDE genotype associated with birth weight in South Asians

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Within an obesogenic environment the South Asian population have a higher propensity to develop obesity and T2D. SA infants appear to have more central visceral adipose tissue at birth than white Caucasians. Raised levels of adipose tissue increases the risk of T2D and cardiovascular disease in adulthood. Low birth weight followed by rapid catch up to normal weight in babies has been associated with insulin VNTR alleles and childhood obesity. HHEX - IDE is a recently published candidate region for a role in obesity and T2D in Caucasians. There is some evidence that HHEX - IDE influences fetal birth weight with a parent of origin effect. Since insulin is an important intrauterine growth factor, HHEX - IDE is an interesting candidate in either increasing or ameliorating childhood obesity. Our study was designed to ascertain whether variants in the HHEX - IDE locus of 663 SA mothers influence fetal birth weight. Using the Sequenom iPLEX assay, 37 SNPs spanning the maternal HHEX - IDE locus have been genotyped. ANCOVA analysis controlling for gestational age, maternal booking weight, fetal sex and parity revealed one SNP significantly associated with birth weight (rs12765131 $p=0.002$) and two trend associated. Three SNPs were also found to be associated with T2D in the mothers (rs4933236 $p=0.011$, rs2488073 $p=0.020$ and rs2497349

$p=0.016$) with a further two trend associated. This study provides preliminary evidence that variants in the maternal HHEX - IDE locus are associated with birth weight in SA subjects and also contributes to the development of T2D.

P09.066 Serotonin receptor 1A -1019C/G polymorphism may influence treatment response to escitalopram in patients with melancholic depression

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In our previous study we demonstrated that melancholic subtype of depression predicted poor treatment response to escitalopram; however this was not associated with the serotonin transporter promoter-linked polymorphic region polymorphisms. Recently, Baune et al reported that functional polymorphism -1019C/G in serotonin receptor 1A gene may have specific role in mediating antidepressant efficacy in depression with melancholic features. In current study we examined the possible impact of this polymorphism on the treatment response to escitalopram in patients with major depression and particularly in depressive patients with melancholia. The 5-HT1A -1019C/G polymorphism was genotyped in 135 outpatients with major depressive disorder, MDD (mean age 31.1 ± 11.6 years, 68% females) treated with escitalopram 10-20 mg/day for 12 weeks. Overall, there was no significant association between -1019C/G polymorphism and escitalopram efficacy in total sample of patients with MDD diagnosis. However, the melancholic patients carrying GG genotype showed significantly poorer treatment response at week 12 of escitalopram medication than melancholic patients with C alleles. The results of our study may give additional support to importance of 5-HT1A -1019C/G polymorphism in modulation of antidepressant treatment in the melancholic subtype of depression.

P09.067** Genome-Wide Association Study reveals new gene for hypospadias.

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Hypospadias is a common congenital malformation of the male external genitalia with a multifactorial etiology. To identify genetic variants contributing to susceptibility for hypospadias, we performed the first Genome-wide Association Study (GWAS) for this malformation using DNA pooling. We included 436 Caucasian cases with isolated glanular or penile hypospadias from the AGORA project (Aetiologic research into Genetic and Occupational/environmental Risk factors for Anomalies in children) and 494 unaffected Dutch males and allelotyped 906,600 single nucleotide polymorphisms (SNPs) using Affymetrix 6.0 arrays. Allele frequencies were calculated using k-corrected signal intensities. The highest ranked SNPs that fulfilled quality-control criteria were selected for individual genotyping. Seven SNPs were located in one X-chromosomal gene. As most SNPs in this gene are in strong linkage disequilibrium with each other, individual samples were genotyped for the (intronic) SNP that tagged most other SNPs and for a potentially regulatory SNP in the 5' upstream region. Both SNPs showed highly statistically significant association results in individual genotyping (odds ratio (OR) = 2.45, 95% confidence interval (CI) 1.88-3.20, $p=3.2E-11$ and OR=2.25, 95% CI 1.71-2.94, $p=3.5E-9$, respectively). These results were replicated in an additional Dutch sample of 75 hypospadias cases and 102 controls (OR=2.18, 95% CI 1.17-4.05, $p=0.014$ and OR=1.89, 95% CI 1.01-3.53, $p=0.045$, respectively). Furthermore, nine SNPs in other genes were genotyped in the individual samples, of which seven showed statistically significant results ($p<0.05$). Replication in 350 hypospadias cases and 350 controls from Sweden is ongoing as are experiments studying expression of the X-chromosomal gene in relevant human tissue.

P09.068 Distribution of functional polymorphic variants of pro-inflammatory related genes RANTES and CCR5 in long-lived individuals

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The positive effect of the immune system protecting against pathogens and tumour-cells spread, can be turned out, later in life, as a detrimental chronic inflammation fading longevity. It has been considered that the persistent activation of inflammatory status is related to unsuccessful aging meanwhile the prevention of inflammation contributes to healthy aging. Nevertheless, a pro-inflammatory status is the common phenotype in older people. In order to assess for a genetic component in the elderly pro-inflammatory status we have studied the distribution of functional polymorphic variants of two genes from the chemokine pro-inflammatory pathway, RANTES and CCR5, in long-lived individuals. A promoter polymorphism of the RANTES gene (rs2107538; -403G/A) and an insertion/deletion polymorphism of the CCR5 gene (rs333, delta32) were genotyped in a cohort of 214 subjects; 104 octogenarians (>85 years, 72 women and 32 men) and 110 sex-matched healthy young individuals (75 women and 35 men). Selected polymorphic variants have functional effects on the RANTES/CCR5 pro-inflammatory mediated pathway. RANTES-403A rare allele increases the promoter transcriptional activity that results in high serum RANTES levels favouring a pro-inflammatory status. In contrast, delta32 CCR5 deletion variant abrogates functional CCR5 receptor expressions that weaken the pro-inflammatory CCR5 mediated action. No differences were observed in global genotype and allele distribution between octogenarians and controls. In contrast, male octogenarians shows and overrepresentation of RANTES-403A allele (OR=3.2 95% C.I.=1.2-8.5; p=0.012) and RANTES-403A carrier genotypes (OR=4.0 95% C.I.=1.3-11.7; p=0.01). Our results suggest a male-specific pro-inflammatory genetic determinant that could contribute to the known gender differences in aging.

P09.069 Oxidative and carbonyl stress-induced DNA damage in patients with inflammatory bowel disease

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Crohn's disease (CD) and ulcerative colitis (UC) are chronic relapsing inflammatory disorders of the gastrointestinal tract. Oxidative stress, defined as an imbalance between the production of reactive oxygen species and antioxidative mechanisms, caused by the chronic inflammation is one of the potential mechanisms leading to systemic complications. Mitochondria are organelles with the highest production of free radicals and lowest antioxidant status, therefore, mitochondrial DNA (mtDNA) is at high risk of oxidative damage. Increased oxidative stress in inflammatory bowel diseases (IBD) leads to lipoperoxidation and the production of carbonyl compounds. Carbonyl stress represents an important pathomechanism in several metabolic diseases, but its role in IBD has not been described yet.

We investigated 212 IBD patients and 47 control subjects. In our study we analyzed the fragmentation of mtDNA in association with polymorphisms in *ATG16L1* and *PTGER4* and carbonyl damage of DNA quantified as AGE-specific fluorescence.

In our cohort of Slovak IBD patients we did not confirm the association of rs3828309 in *ATG16L1* and rs4613763 in *PTGER4* with IBD described in several genome-wide association studies. We did not find any differences in AGE-specific fluorescence of DNA between IBD patients and controls. In contrast, fragmentation of mtDNA was found to be increased in CD patients compared to controls depending on the *ATG16L1* genotype.

Our findings should be complemented by analyzing additional biochemical markers of oxidative and carbonyl stress in further studies.

P09.070 Detection, genetic impact and distribution of hypervariable region in human Na(+)-Ca(2+) exchanger (NCX1) intronic region among European, Asian and African populations

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Although short insertions and deletions (indels; <100bp) are abundant in the human genome, little attention is paid to their effect on gene regulation and disease susceptibility. We have targeted non-coding regions of cardiovascular disease (CVD) candidate gene, Na(+)-Ca(2+) exchanger (NCX1) with polymorphism screening among CVD patients and identified a 14bp intronic indel (rs11274804). Genotyping among Eastern-Europeans (n=1792) revealed that the analyzed 348bp region was hypervariable, represented by seven different alleles. The alignments of human-chimpanzee-macaque sequences showed that the major human variant (allele frequency 90.45%) was actually a human-specific deletion compared to other primates. In humans, this deletion was surrounded by other short (5-43bp) deletion variants and a duplication (40bp) polymorphism possessing overlapping breakpoints. The observed high variation reflects on a potential indel hotspot, which may have been triggered by the initial deletion in human lineage. Association of NCX1 intronic 14bp indel (rs11274804) with CVD was studied in two Eastern-European sample sets: essential hypertension (HYPEST; n=1122; cases n=470/controls=652) and coronary artery disease, CAD (CADCZ; n=670; cases n=257/controls=413). An association was detected between the carrier status of 14bp indel allele and the diagnosis of CAD (P=0.0016, OR=2.02; Bonferroni significance level α =0.0045), but not with hypertension. The risk for the CAD development was even higher among the patients additionally diagnosed with metabolic syndrome (P=0.0014, OR=2.34). Consistent with the effect on metabolic processes, the 14bp indel showed also suggestive evidence for the association with heart rate, serum triglyceride and LDL levels (P=0.04).

P09.071 Association between genetic variation in the gene encoding coagulation factor XI and ischemic stroke

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Background: Coagulation factor XI (FXI) contributes to hemostasis by activating factor IX and by down-regulating fibrinolysis. Association between high levels of FXI and atherothrombotic disease including ischemic stroke (IS) has been reported in a few studies. The aim of the present study was to investigate whether there is an association between genetic variation in the gene encoding FXI (*F11*) and IS.

Methods: The Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) comprises 845 Caucasian patients with IS and 668 Caucasian population-based controls. Stroke subtype was defined using TOAST criteria. Seven tagSNPs in the *F11* gene were selected from HapMap. Genotyping was performed with the Illumina GoldenGate assay. Haplotypes were inferred using the EM algorithm.

Results: Three SNPs (rs1593, rs3733440, and rs925451) in the *F11* gene showed significant associations with overall IS. All associations remained after adjustment for vascular risk factors with odds ratios (ORs) for the minor allele of 0.69 (95% confidence interval (CI) 0.54-0.88), 0.76 (95% CI 0.60-0.96) and 1.24 (95% CI 1.06-1.46), respectively. Genetic variation in the *F11* gene was also independently associated with the etiologic IS subtypes of small-vessel disease (SVD), cardioembolic (CE) stroke and cryptogenic stroke. Furthermore, haplotype analysis revealed that two *F11* haplotypes were significantly associated with overall IS.

Conclusions: In this study we found independent associations between *F11* gene variation and overall IS as well as the IS subtypes of SVD, CE stroke, and cryptogenic stroke. These findings motivate further studies on the importance of FXI in IS.

P09.072 Genetic variation on chromosome 9p21 shows association with large-vessel disease in a Swedish sample aged ≤70 years

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Background: Ischemic stroke (IS) is a complex disease in which environmental and genetic factors make about equal contribution to the etiology. The aim of this study was to independently validate a recent finding of an association between genetic variants on chromosome 9p21 and the subtype large-vessel disease (LVD).

Methods: The Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) comprises 844 Caucasian patients, who suffered IS before reaching the age of 70 years, and 668 healthy Caucasian controls. IS subtype was defined according to the TOAST criteria, and 111 patients were categorized as LVD. Seven SNPs, rs10965227, rs1547705, rs1333040, rs7857345, rs1333045, rs10757278, and rs1537378 were analyzed.

Results: The mean age of controls and patients with IS (including LVD) was 56 years and the mean age of patients with LVD was 60 years. The uncommon (A) allele of SNP rs7857345 was associated with a decreased risk of LVD after adjustment for age and sex (OR 0.61, 95% CI 0.44-0.86, $p=0.004$). This association remained after adjustment also for vascular risk factors (OR 0.58, 95% CI 0.39-0.86, $p=0.007$), and in this analysis an association with LVD was also detected for rs10965227 (OR 1.57, 95% CI 1.06-2.34, $p=0.02$). In contrast, no significant associations were found for the other three IS subtypes or overall IS. In addition, an association between rs7857345 and functional outcome two years after LVD was also observed.

Conclusion: In this relatively young Caucasian population of patients with IS, genetic variation on 9p21 is associated with the IS subtype LVD.

P09.073 Association between connective tissue disorders, bone mineral density and bone metabolism in juvenile idiopathic arthritis patients

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Background and aims: Connective tissue disorders (CTD) are very common clinical syndrome among children with juvenile idiopathic arthritis (JIA). The aim of our study was to investigate and compare data on bone densitometry, bone biochemistry and molecular assay in JIA patients depending on present of CTD.

Methods: 198 JIA children were included in our study. P. Beighton score was used for determinate joint hypermobility index. Bone mineralization was detected by dual-energy X-ray absorbiometry of lumbar spine. Bone metabolic parameters such as osteocalcine, parathyroid hormone and C-terminal telopeptides, Ca and Ca²⁺, nonorganic phosphorus levels and total alkaline phosphatase (TAP) activity were determined. Molecular testing of *Apal*, *Tagl*, *BsmI* *VDR* gene polymorphisms, *Sp1* *Col1a1* gene polymorphism and *HindIII* *BGLAP* gene polymorphism were provided by PCR-RFLP.

Results: We have revealed that CTD was more frequent among JIA patients (79.7%). Low bone mineral density (BMD) for chronological age was 3 times higher in CTD children with JIA, in boys also higher (26.7%) than in girls (20.0%). Significantly lower BMD (-0.19±1.05 SD and 0.5±0.92 SD, relatively, $p=0.01$) and higher TAP activity (354.6 ±117.2 UI and 300.1±102.1 UI, respectively, $p=0.05$) were detected in CTD children. There was no difference in genotype and allelic distribution of studied genes, impacted in bone metabolism and mineralization in JIA children, depending on presence of CTD.

Conclusion: We have revealed an association between CTD and low bone mineralization, high alkaline phosphatase activity and found no difference in frequencies of *VDR* and *Col1a1* genetic polymorphisms in children with JIA.

P09.074 Low frequency variants affecting lipid levels in the Finnish population

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The SNP genotyping platforms for genome-wide association studies (GWASs) contain mostly common SNPs, but it has become evident that common SNPs only explain a part of the heritability of complex disorders. A small number of low frequency SNPs (lfSNPs) are also present on these chips, but the statistical power to detect association between a single low frequency variant and a phenotype is low. The presence or absence of lfSNPs within a predefined region can be used as a pseudomarker which provides increased statistical power to identify genes with multiple low frequency variants contributing to the phenotype. Although these rare variant collapsing methods are primarily developed for resequencing data, here we have used available genome-wide SNP data to test for association of low frequency variants with lipid measures.

We used three Finnish datasets (n=8913) with measures of total cholesterol, HDL, LDL and triglyceride levels. We used QuTie software, which pools low frequency variants within genes and tests for differences in quantitative trait means in individuals with at least one lfSNP compared to individuals with none. Summary statistics per gene were combined across datasets using inverse variance meta-analysis.

In our preliminary analysis, we identified significant enrichment of lfSNPs in multiple regions, including 20q13.12 and 6p22.2. Next, the genotyping quality of the identified SNPs needs to be addressed. Our results suggest that low frequency variants, which are usually excluded from GWASs, should be included in analyses. Careful inspection of the clustering quality of identified SNPs is needed to exclude false positive signals.

P09.075 Patogenetics of the liver cirrhosis: Polymorphism of glutation S-transferase genes

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Deletion polymorphism in genes *GSTT1*, *GSTM1* and polymorphic variant in gene *GSTP1* (A313G) were studied for their association with cirrhosis and probability of survival during four years from the moment of inclusion into study. Population of Tomsk region (West Siberia) was examined in the current study. We have shown that homozygous deletion for *GSTM1* (null genotype) is protective factor against development of alcoholic and mixed (HCV, HBV and alcohol) liver cirrhosis. The patients in general group and with alcoholic and mixed cirrhosis had lower frequency of null genotypes for *GSTM1* then the control group (39.2%; 39%; 34.2%) ($p=0.6\times10^{-4}$; $p=0.1\times10^{-2}$; $p=0.1\times10^{-3}$). The null genotypes for *GSTM1* and polymorphic variant in *GSTP1*(A313G) were associated with four years patients survival. The survived patients had higher frequency of null genotypes for *GSTM1* (46.6%; 30.2%) ($p=0.03$) and genotypes AA in *GSTP1*(A313G) (63.1% and 40.5%) and lower frequency of genotypes AG in gene *GSTP1* (A313G) (31.1% and 51.2%) ($p=0.8\times10^{-2}$). Chance to survive was higher for patients with genotype AA in *GSTP1* (A313G) ($OR=2.52$; $1.34 < OR < 4.75$; $p=0.3\times10^{-2}$) and null genotypes for *GSTM1* ($OR=2.01$; $1.06 < OR < 3.85$; $p=0.032$). The probability of a fatal outcome is higher for patients with genotype AA in *GSTP1*(A313G) ($OR=2.33$; $1.23 < OR < 4.43$; $p=0.8\times10^{-2}$).

P09.076 Resequencing candidate genes for major depressive disorder in a Dutch cohort

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Major depressive disorder (MDD) is a common complex trait characterized by prolonged dysphoria, changes in appetite and sleeping behaviour, feelings of worthlessness and additional cognitive symptoms. Besides being a burden for patients and their relatives, MDD is also one of the leading causes of disability in western civilization, with enormous consequences for the economy and healthcare.

In a 2008 genome wide association study (GWAS) for MDD, Sullivan et al. found a significant single nucleotide polymorphism (SNP) in the

piccolo gene (pclo). Pclo codes for a protein that is localized in the presynaptic area.

We plan to resequence pclo in 50 control samples to look at all normal variants, find possible new risk factors for MDD, refine patterns of inheritance and to find further evidence that the SNP found by Sullivan et al. is truly the causal SNP. The variations that we want to find are SNPs, Copy Number Variations and Insertions/Deletions.

We hybridize genomic DNA to NimbleGen Sequence Capture arrays to capture the DNA of interest. Hybridized DNA is eluted using NaOH and then amplified with ligation mediated PCR. After amplification we compare the enrichment of our target DNA to genomic DNA for several control loci. After having sufficient enrichment (>100x), indexes are ligated to the DNA for identification. Samples are then sequenced using an Illumina GAIi-machine. Sequence data is then aligned back to the reference genome and variants are detected by CLC Genomics Workbench software.

P09.077 Complex etiology of an apparently Mendelian form of mental retardation

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The genetic causes for an apparently recessive form of non syndromic mental retardation, in a large nordern swedish pedigree from an inbred population, are investigated.

After extensive evaluation of the patients, which ruled out a recognizable pattern of malformation and excluded the most common causes of MR, a comprehensive linkage-wide genome analysis, with 500 microsatellite markers, was performed in 24 members of this family, including 9 mild mentally retarded individuals and 14 non affected individuals. No significant LOD score was found with either parametric and non parametric linkage analysis. The highest scores, both with MODSCORE and SimWalk parametric analysis are located at chromosomes 13, 15 and 17. Additionally, a copy number analysis, using an affymetrix 250K SNP chip, was performed in this pedigree. No clear cause for the disorder was identified; but rather, several variants were present in the family members, irrespective of their affected status.

These results suggest that the mental retardation in this family, unlikely what we were expecting, has a heterogeneous etiology; and that there might be several lower effect genes variants

P09.078 Polymorphic genetic markers play role in developing of the metabolic syndrome abnormalities in men

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We investigated genotype distributions of several polymorphisms of genes APOA1 G-75A and C+83T, APOC3 Sst1, APOE, APOA5 T-1131C and S19W, ADRB3 W64R, and ACE I/D to define predictive genetic factors of metabolic and physiological abnormalities of the metabolic syndrome (MS). Study population: 501 MS patients (176 males and 325 females, average age 61.7±1.0) and 199 controls (115 males of average age 40.0±0.5 and 84 females of average age 85.9±0.5). Almost all MS patients were moderately or severely obese, 382 patients had diabetes mellitus type II. Results. The male patients had a higher rate of APOA5 19W-allele carriers compared to the controls (OR=4.77, 95% CI 1.40-16.24). The males with abdominal obesity had a higher rate of APOA1 -75A-allele (OR=2.01, 95% CI 1.12-3.61) carriers and the males without hypertension had a higher rate of APOE E2-allele carriers (OR=3.16, 95% CI 1.36-7.35), compared to the controls. Also, the rate of APOE E2-allele carriers was higher among the patients with triglyceride levels above the 90th age and sex specific percentile (OR=2.22, 95% CI 1.10-4.48) and among the patients with total cholesterol levels below the 10th age and sex specific percentile (OR=2.47, 95% CI 1.04-5.85), compared to the controls. The only significant difference in female groups was that the patients had a lower rate of ACE D-allele carriers compared to the controls (OR=0.85, 95% CI 0.75-0.97). Females had not a significant difference in genotype

distributions depended on the MS anomalies. Conclusion: Genetic polymorphisms can be associated with some abnormalities of MS in males.

P09.079 A genome-wide perspective of genetic variation in human metabolism

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Serum metabolite concentrations provide a direct readout of biological processes in the human body, and are associated with disorders such as cardiovascular and metabolic diseases. Here we present a genome-wide association study with 163 metabolic traits using 1,809 participants from the KORA population, followed up in the TwinsUK cohort with 422 participants. In eight out of nine replicated loci (FADS1, ELOVL2, ACADS, ACADM, ACADL, SPTLC3, ETFDH, SLC16A9) the genetic variant is located in or near enzyme or solute carrier coding genes, where the associating metabolic traits match the proteins' function. Many of these loci are located in rate limiting steps of important enzymatic reactions. Use of metabolite concentration ratios as proxies for enzymatic reaction rates reduces the variance and yields robust statistical associations with p-values between

3×10^{-24} and 6.5×10^{-179} . These loci explained 5.6% to 36.3% of the observed variance. For three loci, an association with clinical endpoints has previously been reported (SLC22A4 with Crohn's disease, FADS1 with hyperactivity and lipid levels, ACADS as susceptibility locus for ethylmalonic aciduria). For several others (ACADM, ACADL and ETFDH), loss of function of the corresponding gene leads to severe Mendelian disorders. As pointed out in a recent editorial, this shows the potential for discovering key steps in human metabolism, including common but minor inborn errors or minor variations of metabolism that are part of the disease process (Mootha, V.K. and Hirschhorn, J.N. Inborn variation in metabolism. in Nat Genet. 42 97-8 (2010)).

P09.080 Matrix metalloproteinase-9 gene polymorphisms and multiple sclerosis

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To investigate the role of the matrix metalloproteinase-9 gene (MMP-9) in multiple sclerosis (MS), we analyzed the functional -1562C/T and -90 (CA)_n repeat polymorphisms, located in the promoter region of this gene, in 243 Italian MS patients and 173 healthy controls. A markedly significant increase of the -1562T allele carriers in MS patients compared to controls was found ($P = 5.6 \times 10^{-5}$, adjusted OR = 2.57, 95% CI = 1.52-4.36). Moreover, also carriers of higher than 20 CA repeats were significantly more frequent among MS patients than among controls ($P = 0.011$, adjusted OR = 1.53, 95% CI = 1.06-2.02). There were no differences in MS disability status, age at onset and disease duration among the genotypes. Haplotype analysis showed that the haplotype formed by the -1562T allele and the L allele (lower than 20 CA repeats) was over-represented in MS patients versus con-

trols, suggesting it could be a possible risk factor for the disease ($P = 2.14 \times 10^{-5}$, OR=6.79, 95% CI=2.82-16.35). These results suggest that genetic polymorphisms of the *MMP-9* promoter regions may influence the susceptibility to MS.

P09.081 Monoamine oxidase A and B genes (*MAOA* and *MAOB*) and their influence on personality traits in healthy individuals

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Complex phenotypes, such as personality traits, are presumably affected by the interaction of multiple genes of small effect. Since psychobiological model of personality was proposed by Cloninger, interest of scientific groups has been focused on neurotransmitter system genes.

We aimed to define a single genotype effect of *MAOA* VNTR and *MAOB* rs6651806 polymorphisms and to check possible interaction effect between them and personality traits (assessed with TCI-125 questionnaire).

We recruited 652 healthy individuals (men-222, women-430) of Caucasian origin (Russians-233, Tatars-419) from Russia (mean age: 19.53 ± 2.24 years). The main and interaction effects of gene polymorphisms on personality traits were detected under ANOVA and MANOVA (SPSS 13.0).

Considering *MAOA* and *MAOB* genes location, we conducted analysis in each gender separately. Moreover, our preliminary study demonstrated ethnic differences in personality that indicates the necessity of groups' combination with respect to ethnicity. ANOVA performed in female Tatars group revealed an association between *MAOA* 4R-allele and higher Persistence ($P=0.023$). We observed significantly higher Reward Dependence ($P=0.018$) and lower Novelty Seeking ($P=0.007$) in Russian male and female carriers with *MAOB* C-allele respectively. Subsequent MANOVA revealed the influence of *MAOB***MAOA* interaction on Persistence ($P=0.021$) and Cooperativeness ($P=0.015$) in Tatar females, and on Reward Dependence ($P=0.004$) and Cooperativeness ($P=0.001$) in Russian females.

Our findings point to the possible involvement of enzymes responsible for neurotransmitters degradation (*MAOB*, *MAOA*) in approach-related personality traits in healthy individuals.

P09.082 Association of a melatonin receptor 1B (*MTNR1B*) gene variant with fasting glucose and HOMA-B in children and adolescents of high BMI-SDS groups

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Background: Genome-wide association studies have shown, that the polymorphism rs10830963 within the melatonin receptor 1B (*MTNR1B*) gene is strongly associated with fasting glucose levels and the risk of type 2 diabetes. However, these data are limited to adults and little is known whether similar associations are prevalent in overweight and obese children and adolescents as well.

Objective: The aim of our study was to replicate the findings concerning fasting glucose in a sample of 472 overweight and obese children and adolescents (mean body mass index standard deviation score (BMI-SDS): $2.77 (\pm 0.55)$ / mean age: $14 (\pm 2)$ years), who participated in a short-term weight loss program (energy reduction, increased physical activity, behaviour therapy). Furthermore, we investigated an association between genotype and fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR), beta-cell function (HOMA-B), and weight loss as well as changes of glucose during weight loss.

Results: The minor G allele of polymorphism rs10830963 was associated with an increase of fasting glucose levels (0.132 mmol/l , $p=6.37 \times 10^{-5}$) and decreased HOMA-B (-0.233 , $p=1.95 \times 10^{-4}$). BMI-SDS adjustment did not change this association. Categorizing the sample into BMI-SDS groups, the significant association was abolished in children with BMI-SDS < 2.5 . However, for more obese children (BMI-SDS: $2.5 \leq x < 3$) the beta estimate was much higher (0.212 mmol/l , $p=1.26 \times 10^{-5}$).

Conclusion: This is the first study replicating the association between the *MTNR1B* locus and diabetes-related traits in overweight and obese children and adolescents. The effect size in children is higher than in adults, whereas the effect size differs between BMI-SDS categories.

P09.083

Role of Mitochondria in Multiple Sclerosis

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Multiple sclerosis (MS) is a demyelinating disease of the central nervous system characterized by the morphological hallmarks of inflammation, demyelination and axonal loss.

We have sequenced the mtDNA HVS-I in 54 MS patients and 100 control subjects. We have found that haplogroups A and K are significantly more abundant in MS patients. Thus, these two haplogroups might act synergistically to increase the penetrance of MS disease.

Kinetic analysis of mitochondrial respiratory chain complex I enzyme was performed on intact mitochondria isolated from fresh skeletal muscle in MS patients ($n=10$) and control subjects ($n=11$). Mitochondrial DNA common deletion and deletions were also tested in MS patients. Our findings showed that complex I activities were significantly reduced in patients compared with control. However, we could not find deletion in mtDNA of patients with MS. This study suggested that a biochemical defect in complex I activity may be involved in pathogenesis of MS.

To evaluate the link between MS and LHON primary point mutations, we investigated 31 non-related Iranian clinically definite MS patients with optic nerve involvement, as well as 25 patients without involvement of the optic nerve as controls. Our results suggest that there is no association between Iranian patients with MS and mtDNA point mutations at np 11,778, 3,460, and 14,484.

We sequenced mitochondrial complex I subunit in MS patient (ND1-ND6). No pathogenic mutations were found in our patient's. We conclude that it may mitochondrial nuclear subunit of complex I gene play roll in MS patient.

P09.084 CASP-9 exon 1 polymorphism in patients with Multiple Sclerosis.

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The pathogenesis of Multiple Sclerosis (MS) is under strong genetic control involving several genes. Apoptosis signaling-related genes are strong candidate genes for involvement in MS, because apoptosis is a common regulatory mechanism for normal development and homeostasis of the immune system, and the elimination of autoreactive T cells via apoptosis appears to be impaired in MS. Particularly, CASP-9 is an initiator CASP in the apotosome-driven apoptosis pathway and plays a crucial role in the initiation phase of the intrinsic apoptosis pathway. To explore the possibility that the genetic alterations of CASP-9 might be involved in the development of MS, we analyzed a SNP in a set of 296 MS patients from Southern Italy and 255 healthy controls subjects from the same geographical area: a C to T polymorphism in the codon region of the gene at codon 93 (exon 1). Genomic DNA was isolated from blood and this polymorphism was determined by PCR-RFLP assay. Our results showed no significant differences in the allele and genotype distribution of the CASP-9 polymorphism between MS patients and controls. In addition, dividing patients according to disease type, EDSS, or age and gender did not yield significant differences for the examined polymorphism. The present findings from Italian population suggest that there was no association between this polymorphism and susceptibility to MS. Therefore, in the absence of conclusive data, we cannot exclude the possibility that our results are only partially indicative because the association between genetic polymorphisms and MS may also vary with ethnicity.

P09.085 complex I deficiency in MS caused by mitochondrial genes or nuclear one ?

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Multiple sclerosis (MS) is a chronic disease of central nervous system. Demyelination and devastation of axons occur in this disease. Genetic and environmental factors are important in the risk of developing the disease. Complex I that composed of at least 46 subunits contains seven subunits which are encoded by the mtDNA[subunit ND1-2-3-4-4L-5 and 6] plus at least 39 nuclear-encoded subunits complex I. About MS, biochemical studies have established that catalytic activity of complex I has significantly decreased in MS patients compared with control subjects. To study relationship between MS patients and mtDNA-encoded complex I genes we sequenced from 15 representative Iranian MS patients and 10 control. Our results showed different mutations in ND2, ND3, ND4, ND5 and ND6 as following: One patient had a mutation in the ND2 gene at nt5153 A>G, two patients show a mutation in ND3 gene at nt10142 C>T. In ND4 one patient had a mutation at nt11215 C>T, one patient had a change at nt11353 T>C, three patients had a mutation at nt11935 T>C. we found a new mutation in two patients at nt12062 C>T in ND4 gene. Eleven patients had a mutation in ND5 gene at nt12662 A>G. ND6 had a mutation at nt14179 G>A in one patients and new mutation at nt14263 T>C in another patient. We couldn't find any mutation in ND1 and ND4L genes. Even some new mutations in mitochondrial DNA were found in this study we conclude that it may mitochondrial nuclear subunit I may roll in complex I deficiency in MS patients.

P09.086 Repulsive guidance molecule A (RGMA) and interleukin 21 receptor (IL21R) show association with multiple sclerosis

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Background: Multiple sclerosis (MS) is a complex trait which is a chronic inflammatory disease in which a dysregulated immune response directed against myelin antigens causes demyelination and axonal injury the central nervous system. Rat chromosome one harbors overlapping quantitative trait loci (QTL) for cytokine production and experimental models of inflammatory diseases. Analysis in Advanced Intercross Lines of (MOG)-induced experimental autoimmune encephalomyelitis (EAE) resolved the region in two narrow QTL, *Eae30* and *Eae31* including the *RGMA*, *IL21R* and *IL4R* genes.

Hypothesis: Are the same genes that have been implicated in EAE susceptibility on rat chromosome 1 also risk genes for MS?

Materials and Methods: *RGMA*, *IL21R* and *IL4R* were covered with 97 tagSNPs which were genotyped in 1018 MS cases and 1215 controls from Sweden. Markers (n=17) in haplotypes that showed evidence of association were further tested in 2353 cases and 1770 controls from Scandinavia.

Results: In the combined patient material the rs34925346 SNP in *RGMA* was associated with MS in males (OR=1.33, p<0.006). In *IL21R*, The rs8060368-rs2214537-rs961914-rs12934152 CGCT haplotype was positively associated (OR 1.14, p<0.0009) with MS while the TCCC and TGCT haplotypes were negatively associated with MS (OR 0.87, p<0.004, and OR 0.68). One of the protective haplotypes correlated to lower IFN- γ expression in PBMCs of MS patients. We could not confirm previously reported associations between *IL4R* and MS.

Conclusion: We conclude that *RGMA* and *IL21R* and their pathways are crucial in MS pathogenesis and warrant further studies as potential biomarkers and therapeutic targets.

P09.087 The double-faced association of the PRKCA gene with multiple sclerosis

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Multiple sclerosis (MS) is a complex autoimmune disease of the central nervous system characterized by chronic inflammation and progressive neurological dysfunction. The Protein Kinase C Alpha (PRKCA) gene, spanning 508 kb on 17q24.2, was associated with MS in Finnish, Canadian, and UK populations, in which specific risk haplotypes were identified.

In this study, we analyzed the role of PRKCA in MS susceptibility in an Italian cohort of 358 cases and 662 controls. An association analysis was performed by genotyping 3 microsatellites and 20 single nucleotide polymorphisms (SNPs) covering the whole gene.

Single-marker analysis demonstrated a nominal evidence of association for one allele of the microsatellite mapping in the promoter region of the gene, showing a protective effect (P=0.033; OR=0.12, 95% CI=0.015-0.94). Moreover, a risk haplotype composed of 7 SNPs, spanning a 43-kb-long region of PRKCA, was found (P=0.00074; OR=1.57, 95% CI=1.24-1.99). Interestingly, this haplotype includes an exon characterizing an alternative PRKCA transcript. The expression of both PRKCA wild-type and of its alternative isoform was significantly down-regulated in peripheral blood mononuclear cells of MS patients compared to controls (P=0.00097 and P=0.044, respectively). However, stratification of expression data according to the presence/absence of the risk haplotype did not reveal any significant difference, suggesting that a fine mapping of the 43-kb region is needed to identify the actual risk variant.

In conclusion, the identification of both protective and risk alleles in different regions of PRKCA suggests the existence of multiple mechanisms linking genetic variations in this gene to the pathogenesis of MS.

P09.088 Replication study of Multiple sclerosis susceptibility alleles in Austrian patients

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system, thought to be mediated by an autoimmune process. The disease affects over 2 million individuals world wide. Population and family based studies have suggested that there is a strong genetic component. In recent genome-wide and candidate gene-association studies a number of putative MS susceptibility genes were identified. Here we performed a replication study in 883 Austrian MS cases and 996 controls for 26 risk associated loci (39 SNPs). Eight non-human leukocyte antigen (HLA) loci, were associated with disease susceptibility with nominal significant p-values < 0.05: EVI5, RPL5/FAM69A, ASAP2, IL7R, ANKRD15, TBC1D2, IL2RA and CLEC16A. While some loci had been well established before the present study, others such as ASAP2 and TBC1D2 were replicated here for the first time. In an attempt to define a biological consequence of risk variants we mined associated SNPs from our study with data from a recently performed Expression Quantitative Trait loci eQTL study [Heinzen et al]. In this work, genome wide genotyping and ge-

ome wide expression profiling was performed in 93 human brain and 80 blood samples. We revealed a strong association between the risk allele C of the RPL5 SNP rs6604026 and expression of the non coding small nucleolar gene SNORA66, located in intron 7 of RPL5 ($p<2.3\times 10^{-9}$). The discovery and validation of new genetic risk factors in independent populations may help toward the understanding of MS pathogenesis by providing valuable information on biological pathways to be investigated.

P09.089** Searching for functional variants in STAT3-region in Finnish multiple sclerosis patients

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Multiple sclerosis (MS) is considered to be a complex autoimmune disease of the central nervous system. Prevalence of MS in the Southern Ostrobothnia (SO) region in Finland is 2-fold compared to other populations of Northern European descent. We conducted a GWAS in distantly related SO MS patients using genealogical information and identified variants in STAT3 to associate with MS. This association was replicated in a sample set of 4638 cases and 10279 controls from 7 populations (rs744166 p 2.75 x 10E-10).

To refine our understanding of the association and to identify potential functional variants we targeted 111 kb of the STAT3-STAT5A region using SureSelect target enrichment method and Illumina GAII -sequencing. Ten MS samples were chosen based on the homozygous genotype for associated allele of rs744166 and pooled. In total 34 MS samples in four pools and 19 controls in 3 pools were sequenced. The median sequence coverage of 31 was obtained and the preliminary analysis identified 303 unreported variants in the case pool homozygous for the rs744166 risk allele. Twenty of them are missense or nonsense mutations. None of the variants were found in all pooled MS cases homozygous for rs744166, but 13 were specific to the MS pool. In addition to validating the mutations we are currently analyzing expression of STAT3 and near-by genes in PBMCs of 30 MS and 25 control samples to monitor if any of the genes are differentially expressed and if the expression correlates with the genotypes of the associated variants.

P09.090 Ile587Val Polymorphism of the eIF2B5 gene as susceptibility factor for multiple sclerosis

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Multiple sclerosis (MS) is an inflammatory chronic disease characterized by a demyelinating process, which is followed by neurodegeneration. Although the true etiology remains elusive, a lot of evidence have provided that genetic factors contribute to disease susceptibility. Mutations in the eukaryotic translation initiation factor (eIF2B) gene inherited as autosomal recessive trait cause the Vanishing White Matter disease (VWM). MRIs of patients with VWM show a diffuse leukoencephalopathy with evidence of progressive disappearance of the cerebral white matter. Genetic and biochemical data of MS patient and MRI data showing VWM images similar to MS lesions, encouraged the present study in which we analyzed the coding region of eIF2B5 gene to evaluate an overlapping between MS and VWM. We analyzed 225 unrelated MS patients, all of them diagnosed as clinically established, definite MS, according to Poser criteria and derived from the Southern Italy too. More, 207 age- and sex-matched healthy subjects from the same geographic area were included. A common variation Ile587Val was found very frequent in the MS patients respect normal controls.

A statistical analysis (χ^2 -test) suggests that polymorphism Ile587Val in exon 13 of the EIF2B5 gene should be considered as susceptibility factor in the development of MS ($\chi^2=23.76$; $p<0.001$). In conclusion, our data strongly suggest a possible involvement of the eIF2B5 in the development of MS and that variations in the MS patient strongly depend on geographic and ethnic factors, thus suggesting the importance of knowledge the epigenetic contribution in the pathogenesis of MS.

P09.091 Is there a common evolutionary background for music and language faculties? Analysis of dyslexia candidate genes in Finnish families tested for musical aptitude

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Musical aptitude is a cognitive function of the human brain. Common evolutionary background of music and language recognition has been long speculated, based on for example on partially overlapping brain regions in PET studies (Brown et al. 2006). Moreover, low rates in a test for musical aptitude, Karma Music test, have been shown to reliably predict dyslexia (Karma 2002). Here phonological awareness, a basic natural property not dependent on learning and defective in dyslexia could be understood as poor auditory structuring ability measured by KMT. Previously, we have carried out a genome-wide linkage analysis for musical aptitude in multigenerational families tested for musical aptitude using auditory structuring ability test (Karma Music test) and Carl Seashores test for pitch (SP) and for time (ST). Evidence for linkage was obtained for several loci, including DYX6, a candidate locus for dyslexia (Fisher et al. 2002), on chr. 18q (Pulli et al 2008).

In order to understand the neurobiological basis of music in human evolution and communication we analyzed previously reported risk alleles of a set of dyslexia susceptibility genes: KIAA0319, DCDC2, DYX1C1, and C2ORF3. Several SNPs were chosen in these loci and genotyped in multigenerational families (N=395). Family-based association analyses (FBAT and QTDT) were used for analyzing the data. Preliminary results

point to SNPs in KIAA0319 and DCDC2, which are located next to each other on chr. 6 with the Seashore Pitch test, with p-value 0.02. Currently, more families are being genotyped, thus, refined results will be presented in the meeting.

P09.092 The role of neurotrophic tyrosine kinase receptor 3 gene (NTRK3) in personality traits variation: focus on the main, haplotypic and GxE effect

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Personality traits are affected by GxE. NTRK3, a key gene in neurotrophin signaling, is located within the region 15q25.3-q26.2 previously demonstrated linkage with anxiety-related disorders that points to its possible role in personality traits.

We aimed to examine the main and haplotypic effect of NTRK3 gene polymorphisms and to observe GxE interaction contributing into personality traits variation in healthy individuals.

Study sample consisted of 652 healthy individuals (men-222, women-430) of Caucasian origin (Russians-233, Tatars-419) from Russia (mean age: 19.53±2.24 years) without any history of psychopathologies that filled in TCI-125 (Cloninger et al., 1993). Genotyping of 34 SNPs in NTRK3 gene and genotypes assignment was performed with SNPlex™ platform and GeneMapper 4.0 (Applied Biosystems) correspondingly. A measure of linkage disequilibrium between markers was obtained under Haplovview 4.1. Statistical analysis was conducted with PLINK v.1.07 with P-value less than 0.0125 considered statistically significant.

We revealed an association of rs744994 T/T-genotype ($P=0.004$) and higher Novelty Seeking (NS). Haplotype analysis performed in four haplotype blocks ($D'>0.7$) separately demonstrated lower NS in T*-C-haplotype carriers (rs7176520, rs744994) ($P=0.007$) and higher Persistence in those bearing A*T*C*G*C*T*T*T haplotype (rs3825885, rs3825884, rs4887205, rs9806762, rs7180942, rs1110306, rs11073767) ($P<0.001$). GxE analysis demonstrated stronger associa-

tion between *NTRK3* and NS or Persistence in the case of interaction with birth.

The region in *NTRK3* gene located between rs7176520 and rs744994 (24 kb within 5'-UTR and intron 2) is involved in NS variation, while the region between rs3825885 and rs11073767 (134 kb within introns 4 and 12) is associated with Persistence.

P09.093 The influence of R229Q polymorphism of the NPHS2 gene on the progression of chronic glomerulonephritis

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Focal segmental glomerulosclerosis (FSGS) is a common cause of nephrotic syndrome (NS). IgA nephropathy (IGAN) is the most common nephropathy in the world. R229Q polymorphism may increase susceptibility to NS in association with second NPHS2 mutation. We have investigated the influence of R229Q polymorphism in NPHS2 gene on progression of FSGS and IGAN.

A total number of 268 Czech patients with FSGS and IGAN (174 males, 94 females) were studied. The diagnosis of FSGS (98 pts) and IGAN (170 pts) was established from renal biopsy. Patients were divided into two groups: 1. 108 pts (71 males, 37 females, mean age 45.4±10.7 years) with end stage renal disease during 5 years since renal biopsy (rapid progressors), 2. 160 pts (103 males, 57 females, mean age 46.5±12.1 years) who had stable clearance of creatinine during 5 years. As a control group, 100 unrelated healthy subjects (50 males, 50 females, mean age 52.4±9.2 years) were used. Genomic DNA was amplified by polymerase chain reaction. Chi-square test was used to compare the distribution of genotypes. Proteinuria was compared by ANOVA test.

The distribution of R229Q polymorphism in FSGS pts was: in rapid progressors 9.1%, in slow progressors 11.1% of heterozygotes. The distribution in IGAN pts was: in rapid progressors 14%, in slow progressors 10.4% of heterozygotes. Proteinuria was not significantly different in R229Q heterozygotes.

To conclude, we excluded significant influence of R229Q polymorphism in the podocin on progression of FSGS and IGAN. Supported by grant project IGA MZ CR NS 9779-4 and VZ 0021620806

P09.094 Association of Genetic Variants in the Apelin Gene to Obesity in French Caucasians

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Apelin is expressed in adipocytes and upregulated in obese mice. Its peptides are secreted by adipocytes in tissue culture and have been detected in the plasma of both mice and humans suggesting a possible role as a hormone involved in the regulation of feeding. As such Apelin is a plausible candidate gene for human polygenic obesity and this study was designed to investigate an association between variation in the Apelin gene and severe obesity.

Sequenom iPLEX assays were designed to genotype thirteen SNPs within the Apelin gene. Subjects were 1533 obese (896 adults with BMI over 40kg/m² and 637 children with a BMI>97th percentile for age and sex) and 1237 non-obese controls, all French Caucasians.

Two SNPs, rs2235307 and rs2281068 were found to be associated with severe obesity ($p = 0.007$ and 0.003) in female children when analysing genotypes. Male and female genotypes were analysed separately as *APLN* is located on the X chromosome. When allele frequencies were analysed in both sexes, one SNP, rs2281068 was found to be associated in children ($p=0.003$). P-values remain significant after taking into account effects of multiple testing using the method of Nyholt ($p<0.017$).

In conclusion we report a novel association of the Apelin gene to obesity. Re-sequencing of the gene and its promoter region is ongoing to locate the causative variant.

P09.095 CORRELATION OF GENOTYPE AND OBESE PHENOTYPE OF FRAGILE X SYNDROME

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The obese phenotype of fragile X syndrome (FXS) is associated with hyperphagia similar to Prader-Willi. Common variants in the fat mass and obesity associated gene (FTO) show evidence for anxiety and increased appetite. We investigated the association of the haplotype block structure of FTO gene with morbid obesity and with endophenotypes in carriers of FMR1 deleterous alleles.

We selected the SNP with the strongest evidence for association in previous studies rs9939609 and rs1477199, rs1861868, rs1477196 located within same and contiguous haplotype blocks. We genotyped 194 patients with severe obesity appeared < 14 years, 19 cases of FXS, 11 premutation carriers and 289 healthy control individuals. TaqMan® Assays were used and the CGG repeat lengths were determined in the ABI 3130 sequencer using GeneMapper software. SNP frequencies and estimated four-SNP haplotype frequency were compared among groups. Genotype-Phenotype correlations was established.

The two haplotypes rs1477199A/rs1861868A/rs1477196G/rs9939609A and AGGA in the FTO gene raised obesity risk with an odds ratio of 1.31 and 3.35 respectively ($p<0.05$) in Spanish population. 9 FMR1-related individuals carried the AAGA combination in heterozygosity and 3 individuals were homozygous. Positive associations with BMI were detected among them and female sex, ↑ age and longer CGG size also predisposed to pathology.

Obesity and anxiety are associated in some FXS patients. The association between genetic variation in the FTO genotype and obesity could increase even more the propensity to overweight in this specific cognitive deficit. Human syndromes as FXS provide novel insights into processes of the hypothalamic satiety centers and obesogenic pathways.

P09.096 A common haplotype in the NAPEPLD gene involved in endocannabinoid synthesis and obesity in a Norwegian population based cohort (the HUNT study)

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Background: Obesity has a strong genetic component which is still largely unknown.

Material and Method: We investigated the association between severe obesity and genetic variation in six selected candidate genes, including three drug related genes: gastric lipase (*LIPF*), cannabinoid receptor 1 (*CNR1*) and N-acyl phosphatidylethanolamine phospholipase D (*NAPEPLD*), and three genes related to inflammation: interleukin 18 (*IL18*), six-transmembrane epithelial antigen of the prostate 4 (*STEAP4*) and nicotinamide phosphoribosyltransferase (*NAMPT*). Tagging single nucleotide polymorphisms (SNPs) of the coding region of these genes were analyzed in 1632 cases ($BMI \geq 35 \text{ kg/m}^2$) and 3379 controls ($BMI 20-24.9 \text{ kg/m}^2$) from a Norwegian population based cohort, the HUNT study.

Results: Single SNPs rs17605251 in *NAPEPLD* and rs7913071 in *LIPF* were associated with obesity at a nominal significance level ($p<0.05$). Haplotype analysis revealed a common haplotype in the *NAPEPLD* that was associated with obesity, even after correction for multiple testing. The allele frequency was 56.8 % in cases and 60.3 % in controls, giving an odds ratio (OR) of 0.87, $p=0.0016$. Being homozygous for this haplotype gives an OR of 0.79, $p=0.00059$.

Conclusion: We identified a common haplotype in *NAPEPLD*, a rate limiting enzyme of the endocannabinoid synthesis. Individuals who are homozygous for this haplotype have an OR of 0.79 for severe obesity, corresponding to a 20% risk reduction. *LIPF* was associated with obesity at a nominal level but did not remain significant after correction for multiple testing. For the remaining genes: *CNR1*, *IL18*, *STEAP4* and *NAMPT* we found no association to obesity.

P09.097 Cryptic chromosomal abnormalities are a common cause of syndromic obesity: Array CGH study and review**

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Chromosomal imbalance causing obesity has previously been recognised in both recurrent 'syndromes' and unique deletions and/or duplications in individual patients. Many of the chromosomal regions identified in this manner have identified 'obesogenic' genes or are loci which have also been highlighted by genome-wide association studies in non-syndromic obesity. We have previously identified chromosomal deletions causing syndromic obesity, including a case suggesting prohormone convertase-2 as a novel cause of obesity. We therefore studied 21 patients with severe childhood-onset obesity, with or without other features, using array comparative genomic hybridization (array CGH) to look for chromosomal imbalance. From this cohort we identified 6 patients with copy number variation, in at least 4 of whom this is likely to represent a significant factor in causing their obesity (deletions at 16p11.2, 15q11.2, and 1q21.1, and a duplication of 22q11.2). 16p11.2 microdeletion has recently been highlighted in a large series of childhood obesity as a recurrent finding, although the causative gene suggested from this (SH2B1) is not deleted in our and other cases of 16p11.2 microdeletion with obesity suggesting further obesogenic gene(s) at this locus. All four loci are associated with a neurobehavioural phenotype and it is likely effects are predominantly through appetite and satiety regulation, a common finding to the majority of single-gene causes of obesity, although alteration of metabolism is possible. This indicates that as a group chromosomal imbalance is a common cause of childhood obesity, being more common than a recognised single gene cause.

P09.098 Association study on HAPLN1 and MMP genes and osteoarthritis

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Background. Osteoarthritis (OA) is a degenerative joint disease common in the elderly and the heritability ranges between 40–60 %. The disease progresses by the breakage of the extracellular matrix of the cartilage formed by collagens, aggrecans and hyaluronic acid. Matrix metalloproteinases (MMP) are a group of enzymes breaking down the collagen network and aggrecans. Our aim was to study the putative role of four MMP genes and hyaluronan and proteoglycan link protein 1 gene (HAPLN1) in OA, all potentially playing a role in the stability of the cartilage.

Methods. We genotyped a total of 37 tagging SNPs covering the MMP3, MMP8, MMP9, MMP13 and HAPLN1 genes in 134 severe familial hand OA cases, 113 unrelated bilateral primary knee OA cases and 2436 population based control samples. The Pseudomarker program was used to monitor for association with individual SNPs and SNP haplotypes.

Results. Eight variants in MMP8, MMP9 and HAPLN1 provided nominal evidence for association with OA ($p < 0.05$). The strongest association signals were observed for rs1940475 in MMP8 in combined OA ($p = 0.008$) and for rs17577 in MMP9 in hand OA ($p = 0.004$).

Discussion. Initial evidence for association was observed between severe OA and the MMP8, MMP9 and HAPLN1 genes. We are in a process of validating the two most significant associations in a population based Finnish Health2000 cohort of 6000 samples.

P09.099 Influence of glutathione-S-reductase gene polymorphisms on bone mineral density and biochemical bone turnover markers

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Glutathione-S-reductase (GSR) regenerates the oxidized form of glutathione that protects human body from negative effects of reactive oxygen species in state of oxidative stress. Recent data suggest that oxidative stress may influence the development of osteoporosis. An aim of our work was to associate two GSR gene tag polymorphisms, located in introns 3 and 10 (A19278G and A45359T), with bone mass density (BMD) and biochemical bone turnover markers on samples of 721 volunteers.

The results showed a statistical significant association between A45359T polymorphism and the age of menopause ($p = 0.038$) in the subgroup of women, BMD of total hip ($p = 0.043$) and lumbar spine ($p = 0.041$) in the subgroup of premenopausal women. A presence of T allele in the genotype has probably a protective impact on BMD. The polymorphism A19278G is statistically significant with lower BMD of neck ($p = 0.034$) and total hip ($p = 0.019$) of women in the range of 60 to 70 years old. In addition, by using a dominant model we have found out that a presence of at least one allele in conjunction with polymorphism is statistically significant with BMD of femoral neck ($p = 0.0017$), total hip ($p = 0.019$) and lumbar spine ($p = 0.010$).

We conclude that there is a need to find out the mechanism, by which these polymorphisms affect age of menopause, bone mineral density and bone turnover markers. However, it is the first time where the association of the polymorphisms in GSR gene with BMD occur.

P09.100 Loci on 1p13, 10p13 and 18q21 are associated with susceptibility to Paget's disease of bone.

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Paget's disease of bone (PDB) is a common disease with a strong genetic component characterised by focal abnormalities of bone turnover in which mutations of *SQSTM1* gene are responsible for ~10% of sporadic and ~40% of familial PDB cases. In this study we sought to identify novel genetic variants that predispose to PDB by genome wide association study. In the discovery stage, we genotyped 750 patients without *SQSTM1* mutations and 1002 controls for 318,237 SNPs using the Illumina platform. Quality control procedures were performed on the data to exclude SNPs and samples with low call rate, low genotype quality score, and non-Caucasian ancestry. Analysis was performed using the Cochran-Mantel-Haenszel test adjusting for population structure. Three loci on 1p13, 10p13, and 18q21 showed genome wide significant association with PDB with ($P < 1.0 \times 10^{-8}$). In the replication stage we genotyped the most significant SNPs ($P < 1 \times 10^{-6}$) from the discovery stage in an independent set of 500 PDB cases and 535 controls using Sequenom iPLEX platform. The combined dataset confirmed the association of variants on 1p13 ($P = 5.4 \times 10^{-24}$; OR=0.55), 10p13 ($P = 6.1 \times 10^{-13}$; OR=0.65), and 18q21 ($P = 5.3 \times 10^{-13}$; OR=1.52) and showed evidence for suggestive association with variants located on 3p24, 8q22, 10q24, and 14q32 ($P < 1 \times 10^{-5}$). As well as advance our understanding of the pathogenesis of PDB, the genetic variants we have identified may have value as diagnostic markers for disease susceptibility and severity. The causal genes and their associated signalling pathways may also represent new targets for the design of new drugs to treat PDB.

P09.101 Tau and alpha-synuclein and genetic susceptibility to Parkinson Disease in the Italian population

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Several loci for familial Parkinson disease (PD) have been described, and over the last years significant efforts have been focused on in-

vestigating the contribution of common variants to PD risk. Recently, 3 independent GWAS have shown a genome-wide significant association of SNPs in the SNCA (alpha-synuclein) and MAPT (Tau) regions. In this frame, we genotyped a large Italian population of 920 PD patients (306 familial, 614 sporadic) and 920 controls for 13 markers located across the SNCA gene, and for 2 SNPs that define the MAPT H1 clade (previously associated with an increased risk of PD). Single-marker analysis demonstrated nominal evidence of association for: i) 5 SNPs in SNCA: 2 SNPs (rs356219, rs356220) in the 3' region associated with increased risk of PD ($P=0.05$, $OR[95\% CI]=1.15[0.99-1.33]$, and $P=0.05$, $OR[95\% CI]=1.15[1.00-1.33]$) and 3 SNPs (rs356186, rs2737029, and rs2197120) located in the intron 4 of the gene, associated with a reduced/increased risk of the disease ($P=4.46E-04$, $OR[95\% CI]=0.74[0.62-0.87]$; $P=0.03$, $OR[95\% CI]=1.16[1.02-1.33]$; and $P=1.05E-03$, $OR[95\% CI]=0.76[0.64-0.89]$); ii) both SNPs (rs1800547, rs9468) identifying the MAPT H1 haplotype ($P=8.13E-04$, $OR[95\% CI]=0.75[0.63-0.89]$; and $P=8.36E-05$, $OR[95\% CI]=0.72[0.61-0.85]$). Moreover, we found a highly significant association between PD and a protective haplotype ($P=7.53E-06$; 14.1% in cases vs 19.9% in controls), spanning 83kb from intron 4 to the 3' end of SNCA. Our findings provide additional evidence of SNCA and MAPT as major PD genes, and underline the concept that, at least in the Italian population, different allelic variants of SNCA (either protective or predisposing) can contribute to PD susceptibility.

P09.102 Increased level of FAS mRNA gene in peripheral blood lymphocytes of patients with LRRK2-associated Parkinson's disease

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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) known today. Although the precise physiological and pathological role of LRRK2 is unclear, direct link between mutant LRRK2 and activation of the extrinsic cell death pathway through FAS receptor has been suggested. The aim of our work was to estimate level of FAS mRNA in peripheral blood lymphocytes (PBL) of patients with LRRK2-associated. The levels of FAS mRNA were estimated in PBL of six patients with LRRK2-linked PD (5 patients with G2019S mutation; 1- V1613A, mean age 65±10), fourteen sporadic PD patients (sPD) without mutations in LRRK2 (mean age 65±8) and nine persons without neurological disorders (controls) (mean age 76±10) using quantitative real-time PCR with TagMan probes. The level of G protein (GNB2L1) mRNA was used as internal control. The level of FAS mRNA was higher in patients with mutations in the LRRK2 gene (4.0±1.0) compared to sPD (0.7±0.4) ($p<0.0001$) and controls (1.1±0.2) ($p<0.01$). mRNA FAS level did not differ between sPD and controls (0.7 vs 1.1, $p=0.26$).

Our results suggest that LRRK2 mutations may lead to the activation of the extrinsic program cell death signaling pathway by means of induction of FAS expression in patients with LRRK2-associated PD.

P09.103 Alpha-synuclein (SNCA) and mRNA SNCA levels in peripheral blood lymphocytes of patients with LRRK2-associated Parkinson's disease

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Mutations in the LRRK2 gene, encoding leucine-rich repeat kinase 2 (LRRK2), is the most frequent cause of familial PD known today. LRRK2-associated PD cases represent classical PD pathology characterized by Lewy bodies, protein deposits found in the neurons. It has been shown that dardarin is co-localized with alpha-synuclein (SNCA), a major component of Lewy bodies. Alpha-synuclein aggregation is implicated in PD pathogenesis. However, the influence of dardarin on alpha-synuclein metabolism and neurodegeneration remains unclear. The aim of the present study was to access alpha-synuclein and SNCA mRNA levels in peripheral blood lymphocytes (PBL) of PD patients with LRRK2 mutations. mRNA SNCA and alpha-synuclein levels were estimated by means of quantitative real-time PCR and western blotting,

correspondently. Patients with LRRK2 mutations (mutations G2019S (n=6); V1613A, (n=2)), patients with sporadic PD (sPD) (n=33) and persons without neurological disorders (controls) (n=18) were taken into analyses. PBL alpha-synuclein levels correlated with mRNA SNCA level (in sPD and controls, $p<0.05$). mRNA SNCA level did not differ between groups while PBL alpha-synuclein was decreased in PD patients with LRRK2 mutations compared with sPD patients without LRRK2 mutations ($p<0.02$) and controls ($p<0.05$). Thus we received the first data about PBL alpha-synuclein in patients with LRRK2 mutations and demonstrated the decreased level of PBL alpha-synuclein in this group along with unchanged level of SNCA gene expression. These results allow us to suggest the influence of abnormal LRRK2 functions, caused by LRRK2 mutations, to unmodified monomeric PBL alpha-synuclein level.

P09.104 Genetic background of primary ciliary dyskinesia in Polish patients

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Five genes known to be involved in PCD pathogenesis were searched for mutations in patients from over 150 Polish families with Kartagener syndrome (KS) and with CDO (ciliary dysfunction only). *DNAH5*. In total, 109 PCD families were screened for the presence of mutations in *DNAH5* exons using SSCP/heteroduplex method. Of the 22 mutations, 3 STOPs, in exons 63, 49 and 34, and one missense in exon 32 (not reported before) were found in more than one family; haplotype background analysis indicated common origin for each of these repetitive mutations. *DNAI1*. The search for mutations in *DNAI1* was conducted in 113 families. The most frequent ones were insertion in intron 1 and missense in exon 17, confirming their previously reported prevalence among European patients. Our study indicates that mutations in *DNAH5* and *DNAI1* are responsible for PCD/KS in at least 15% and 8% families, respectively. *RSPH4A*. A preliminary search for mutations in two of six *RSPH4A* exons revealed the presence of two stop mutations - in exon 1 (1 family) and in exon 3 (in 2 families), a missense mutation (in 1 family) and 4-nt deletion starting at +2 position of intron 3. *RSPH9*. A preliminary search for mutations in three exons revealed a substitution in the 3'UTR (in 1 family). *XL-RPGR*. In one family, a mutation in exon 2 was shown to cause aberrant RNA splicing. In another family, a mutation at 5th position of intron 2 was found to cosegregate with the disease.

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P09.105 A genetic variant of TFAP2B is associated with anthropometric and adiposity related parameters in women with PCOS

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Background: 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. TFAP2B (Transcriptional factor-activating enhancer-binding protein-2 beta) is an adipocyte expressed transcription factor involved in the regulation of adiponectin expression and the development of insulin resistance. This study aimed to evaluate allelic TFAP2B variants for obesity and weight associated parameters in PCOS patients.

Methods: We investigated the effect of an A/G SNP in TFAP2B on hormonal, anthropometric and metabolic parameters in 371 PCOS patients and 133 non PCOS controls.

Results: Genotype frequencies of the TFAP2B SNP did not deviate from Hardy Weinberg equilibrium and were equivalent in PCOS and non PCOS controls. In all PCOS patients and especially in overweight/obese PCOS patients ($BMI \geq 25$), the G allele of TFAP2B was associated with a significantly higher BMI and changes in body fat composition (lean mass, visceral adipose tissue mass). The G allele additionally influenced weight associated parameters and showed significantly higher systolic blood pressure as well as a trend for increased CRP levels.

Conclusion: We demonstrate that variants in the TFAP2B gene influ-

ence anthropometric as well as obesity related parameters in women with PCOS, especially with increased BMI. This might be of high importance for diagnostic and therapeutic aspects in this frequent disease.

P09.106 An erroneous agenda for personalized medicine.

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Ng et al (Nature, 2009) proposed an agenda for predicting multifactorial diseases using the information on DNA associated variants. We argue here that, under the cloak of comparing the performance of two companies, 23andme and Navigenics, Ng et al give credence to their approach with erroneous recommendations.

At the outset, they confound relative risk (RR) and predictive risk. The term "relative risk" has no meaning unless the baseline risk relative to which the risks refer is specified. In many parts of the paper, the term RR refers to the ratio of risk among those who have the allele to that among those who do not. They advise focusing on markers with high RR and consider them as having high-risk prediction. They seem to ignore that large allelic RR can result in a very low predictive value. Furthermore, there is no sense to the recommendation that, when a test is marketed, the consumer should be informed of the proportion of the disease heritability it explains. The heritability of a disease is usually computed as the heritability of a conceptual "liability" that has a completely different distribution under different models. For multifactorial diseases the underlying model is unknown and likely very complex. The computation of a predictive risk (and its confidence interval) should be specific to sub-groups that we cannot define so long as the environmental factors involved are partly or totally unknown.

P Ng, S Murray, S Levy and J Craig Venter: Nature, vol 461/issue no 7265, 8 October 2009

P09.107 The role of sex determination genes in Polycystic Ovary Syndrome

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Polycystic ovary syndrome (PCOS) is a common complex genetic disorder. Its inherited basis was established by studies demonstrating its increased prevalence including hyperandrogenemia, insulin resistance, and disorders of insulin secretion in relatives of women with PCOS. Human homologs of sex determination genes in the nematode *Caenorhabditis elegans* (FEM1A, FEM1B) were proposed as candidate genes for PCOS [Goodarzi et al., 2008, Hum. Reprod.], but this finding has not been replicated.

Methods: We investigated the potential role of FEM1A and FEM1B single nucleotide polymorphisms in PCOS in a case control study (370 affected women with PCOS and 134 healthy controls). Hormonal, metabolic and anthropometric phenotypes have been collected. Genotype frequencies did not deviate from Hardy Weinberg equilibrium and were not different between PCOS and control women.

Results: FEM1B variants were significantly associated with higher CRP levels, even within the normal range. There was a trend in allele-dependent body fat distribution in FEM1A and a significant association with acanthosis nigricans in FEM1B gene variants. Metabolic parameters were not found to be significantly associated with these FEM polymorphisms. To confirm these findings, further investigations will be performed.

Conclusion: PCOS women with certain FEM1B alleles might be predisposed for higher CRP levels and therefore be at higher risk for cardiovascular events than PCOS women without these alleles.

P09.108 Association of SNPs and haplotypes at MTHFR gene with coronary atherosclerosis

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Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme of folate cycle, which catalyzes for the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The reduced activity of this enzyme, often conditioned by the presence of certain MTHFR alleles, leads to increasing homocysteine concentrations in blood. We

focused on 12 SNPs spanning the MTHFR-coding region: rs3753588, rs2066470, rs17037397, rs7533315, rs4846052, rs1801133 (C677T), rs6541003, rs2066462, rs1801131 (A1298C), rs17375901, rs2274976 (G1793A) and rs1537516. The prevalence of the C677T and A1298C genotypes did not differ significantly between 141 individuals with documented coronary artery disease (CAD) and 126 individuals without vascular disease from a Russian population. We detected significant associations for CAD with rs7533315 (OR=1,60; 95% CI: 1,08-2,36), rs2066462 (OR=2,71; 95% CI: 1,13-6,72) and with GCCTTCGCACGC haplotype (OR 2,98, 95% CI 1,53-5,88). Moreover we found one protective haplotype GCCCTCGCCCGC (OR 0,18, 95% CI 0,04-0,69). In the total sample 40 out of 4096 possible haplotypes were found. The majority of genetic diversity is comprised by 4 common haplotypes, which accounted for 71% of all chromosomes. This study suggests that two polymorphisms (rs7533315 and rs2066462) in MTHFR gene should be assessed as new genetic risk factors for CAD.

P09.109 Significant association of LPP and REL genes in Potential CD patients

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A contribution in the pathway of gluten-induced immunoresponse in Celiac Disease (CD) was gained by candidate genes discovered in GWAS. These new understanding could help to elucidate the mechanism through which CD manifests in so many different ways: Potential celiac cases are patients who produce anti-trasglutaminase antibodies, but haven't small intestinal mucosa damage.

643 CD cases, 105 potential CD patients and 711 controls were genotyped for eight of CD-associated SNPs by Taqman technology. Expression studies of LPP and REL genes were studied by Real-Time PCR using intestinal biopsy samples of 10 CD patients, 10 potential CD and 10 healthy controls.

Candidate Gene SNPs typing shows that the genotype of REL gene SNP (rs842647) can differentiate Potential cases from Controls and Celiac patients, and LPP gene SNP (rs1464510) only from controls. Expression studies show that REL gene only was over-expressed in potential patients when compared to controls and CD.

Potential celiac cases are a living model of gradual expression of the phenotype of CD. The different expression of the REL gene, found in potential cases, suggests a probable role in the pathogenic mechanism of the CD.

P09.110 The combined impact of metabolic gene polymorphisms on elite power athlete status

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Power performance is a complex phenotype, subject to the influence of both environmental and genetic factors. Whilst the last decade has seen a variety of specific genetic factors proposed, each is likely to make a limited contribution to an 'elite' phenotype: it seems more likely that such status depends upon the simultaneous presence of multiple such variants. The aim of the present study was to investigate the associations of multiple common metabolic gene (involved in ATP, glucose, insulin and lipid metabolism, mitochondrial biogenesis, thermogenesis, regulation of muscle fiber type composition and angiogenesis) polymorphisms with power athlete status. The study involved 416 Russian power athletes and 696 controls. ACE, AMPD1, HIF1A, NFATC4, PPARA, PPARD, PPARG, PPARGC1A, PPARGC1B, PPP3R1, TFAM, UCP2, UCP3, VEGFA gene polymorphisms were determined by PCR-RLFP. Four 'power alleles' were first identified showing discrete associations with elite power athlete status (HIF1A 582Ser: 15.8% vs 7.8%, P = 0.0054; PPARA rs4253778 C: 19.3% vs 16.4%, P = 0.048; PPARG 12Ala: 19.9% vs 15.3%, P = 0.0017; PPARGC1B 203Pro: 7.4% vs 4.9%, P = 0.017). Next, to assess the combined impact of all 4 gene polymorphisms, all athletes were classified according to the number of 'power' alleles they possessed. The proportion of subjects with a high (2-5) number of 'power' alleles was greater in the best power athletes compared to controls (41.3% vs 25.0%, P = 0.0036). These data suggest that the likelihood of becoming an elite power athlete depends on the carriage of a high number of power-related alleles.

P09.111 Inherited thrombophilia as a risk factor for pre-eclampsia

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The role of inherited thrombophilia in onset and progression of severe complication of pregnancy such as pre-eclampsia still remains obscure. The major goal of the present study was to determine the relationship between inherited thrombophilia and the risk of pre-eclampsia for pregnant women in Russia. The study was carried out in 77 patients with pre-eclampsia and 100 pregnant women as a control. Pattern of tested genes included: factor V Leiden (*F5* 1691G>A), prothrombin gene (*F2* 20210G>A), polymorphisms of factor 7 (*F7* 10976G>A), fibrinogen (*FGB* -455G>A), plasminogen activator inhibitor-1 (*PAI1* -675 5G/4G), glycoprotein IIIa (*ITGB3* 1565T>C), glycoprotein Ia (*ITGA2* 807C>T) and methylenetetrahydrofolate reductase (*MTHFR* 677C>T) genes using biochip technology. The distribution of genotypes frequencies for *F5*, *F2*, *F7*, *FGB* and *ITGB3* genes was not statistically significant in group of pre-eclamptic patients compared to these ones in the controls. But distribution of genotypes frequencies of *PAI1* ($\chi^2=7.365$, $p=0.025$), *ITGA2* ($\chi^2=6.003$, $p=0.049$) and of *MTHFR* ($\chi^2=6.198$, $p=0.045$) genes were different in these groups. Thus inherited thrombophilia might be considered as a risk factor for pre-eclampsia. Pregnant women should be tested for inherited thrombophilia.

The work was partly supported by grant of President of Russian Foundation 2008 (MK-2961.2008.7), grant of Russian Foundation for Basic Research (N 09-04-13847-ofi-c), and grants of Russian Ministry of Science and Education (N 02.512.11.2275 and N P2387)

P09.112 A search for the most reliable biochemical, hormonal, and genetic markers for hypertension in Saudi patients

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This study was conducted to identify presymptomatic markers of hypertension in Saudi. The study included 153 hypertensive patients and (males: 67; females: 90) and 369 controls (males: 162; females: 207). Blood samples, collected from fasting individuals, were analysed for biochemical, immunological and hormonal parameters [renal, bone, liver function tests, electrolytes, lipids and lipoproteins, apolipoproteins, immunoglobulins and complement levels, insulin, C-peptide, coagulation parameters, leptin and Lp(a)]. The DNA was extracted and used for the analysis of angiotensin converting enzyme (ACE) gene, *S_A* gene, glycogen synthetase gene, glucokinase gene, and paraoxonase (PON) gene polymorphisms. The results showed that the frequency of ACE genotype DD was lower (52.17 % vs 56.03%), while that of ID (43.48% vs 41.84%) and II (4.34% vs 2.13%) were slightly higher in the hypertensive patients compared to the control group but the differences were not statistically significant. In addition, D and I allele frequency did not show any significant difference. When the different biochemical, haematological, immunological parameters were compared, diastolic bp was the highest in the II genotype followed by the ID genotype. In addition, HDL levels were lowest in the II genotype while creatinine levels were highest in the DD genotype. For the *S_A* gene, the genotype A1A1 and A2A2 occurred at a higher frequency, while A1A2 occurred at a lower frequency compared to the controls and the difference was statistically significant. The results showed a significant elevation in Lp(a) in the genotype A1A2. The paper will present these results in Saudi hypertensive patients to identify the most reliable markers.

P09.113 Polymorphisms in the FAM167A(C8ORF13)-BLK locus and TNFSF4 (Ox40L) gene are associated with primary Sjögren's syndrome

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The purpose of this candidate gene study was to identify SNPs in genes with a putative role in the pathogenesis of primary Sjögren's syndrome (pSS). Genes from the type I IFN system, genes involved in inflammatory processes and lymphoma development and genes previously shown to be associated with SLE in GWAS were included.

The genetic variations of the selected genes were covered with Tag-SNPs and genotyped in two case/control cohorts from Sweden and Norway, using Illumina GoldenGate assay. After quality filtering, 1121 SNPs in 82 genes and 540 patients and 532 controls remained for the association analysis. Allele counts and genotype frequencies were compared between patients and controls by Fisher's exact test. Combined p-values and OR of the two cohorts were calculated with Cochran-Mantel-Haenzel test. Clinical data was extracted from the patient files.

We found high signal for association between pSS and several SNPs in the FAM167A-BLK region ($p=4.7 \times 10^{-4}$, OR=1.37) and the TNFSF4 gene ($p=7.4 \times 10^{-4}$, OR=1.34), which have not previously been associated with pSS. In addition we found an expected association with the IRF5/TNPO3 locus ($p=5.5 \times 10^{-6}$, OR=1.70) and the STAT4 gene ($p=7.0 \times 10^{-4}$, OR=1.40). We investigated the correlation between the best SNPs in FAM167A-BLK and TNFSF4 and the presence of Raynaud's phenomenon, arthritis, dermal vasculitis, major salivary gland swelling, lymphadenopathy, lymphoma, leucopenia, hypergammaglobulinemia and anti-SSA/SSB antibodies in the patients. No convincing correlation was detected between the SNPs and the phenotypes.

We conclude that genes involved in B cell activation are important in the pathogenesis of pSS.

P09.114 Absence of mutation in the *PRNP* gene and heterozygosity at codon 129 in probands of an Iranian family with two CDJ affected members

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Prion diseases are a group of rare fatal neurodegenerative disorders characterized by accumulation of a host encoded membrane protein and exemplified by Creutzfeldt-Jakob disease (CJD). The causative protein is the prion protein (PrP) entirely encoded within the second exon of the *PRNP* gene. Up to 15% prion disease cases appear to be familial and inheritance is considered autosomal dominant. Variations in the gene sequence resulting in altered amino acids promote the aggregation process and ultimately disease status. More than 60 different pathogenic mutations in *PRNP* have been identified, and the most common cause amino acid changes at positions 102, 178, and 200. Additionally, the variation resulting in methionine/valine polymorphism at position 129 is considered a genetic susceptibility factor for prion diseases. Here, we report the identification of an Iranian family consisting of two CDJ affected siblings both of whom first manifested symptoms in the second decade of their lives. Both exons of *PRNP* were screened for mutations by direct sequencing and a mutation was not observed. Their genotypes predicted heterozygosity (Met/Val) at position 129. This genotype is not expected to promote disease status. To the best of our knowledge, this is the first report of familial incidence of a prion disease without mutation in the *PRNP* gene.

P09.115 ZNF750 is a nuclear protein whose promoter & 5' UTR variants are found in psoriasis patients

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We previously showed that a dominant mutation in the novel zinc finger protein, ZNF750, that is located within PSORS2 locus, causes seborrhea-like dermatosis with psoriasisiform elements. Furthermore, ZNF750 c.-625A>C promoter variant has been linked with familial psoriasis.

riasis in Chinese patients. We now sequenced DNA samples of 250 bone-fide psoriasis patients. No mutations were found in the ZNF750 coding sequence. However, sequence variants in the ZNF750 5'-UTR were found in 3 of 250 unrelated psoriasis patients and not in 300 controls. It is yet unclear how this variation affects ZNF750 expression. To begin to understand ZNF750 function, we determined ZNF750 sub-cellular localization: HEK293 and HaCaT cells were transiently transfected with EGFP-ZNF750 constructs of full-length or of partially truncated protein, abrogating the ZNF750 NLS. Both confocal fluorescence microscopy and cellular fractionation followed by western blot analysis demonstrated that ZNF750 is expressed in the cell nucleus both in HEK293 and HaCaT cells. Removal of the nuclear localization signal of ZNF750 abrogated its nuclear localization.

Our data suggest that ZNF750 5'-UTR variants might be associated with bone fide psoriasis. Although ZNF750 has a single zinc finger C2H2-like motif, its nuclear localization suggests that it might act as a transcription factor.

P09.116 Variants in LD with the LCE gene cluster deletion are associated with susceptibility to psoriatic arthritis

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Objective: A common deletion mapping to the PSORS4 locus on chromosome 1q21 and encompassing two genes of the late cornified envelope (LCE) gene cluster has been associated with increased risk of psoriasis vulgaris (PsV). One previous report found no association of the deletion with psoriatic arthritis (PsA) suggesting it may be a specific risk factor for PsV. Given the genetic overlap between PsA and PsV we have investigated whether SNPs mapping to this locus, and which are in linkage disequilibrium with the deletion, are risk factors for PsA in a UK and Irish population.

Methods: Three SNPs mapping to 1q21 with prior evidence for association with susceptibility to PsV were genotyped in 1057 PsA patients using Sequenom iPLEX chemistry and genotype frequencies compared with data available for 5575 healthy controls. Two of the SNPs, rs4112788 and rs4085613, were reported to be highly correlated with the LCE deletion. The third SNP, rs6701216, was previously reported to be associated with PsV in a US population.

Results: The alleles tagging the deletion for both rs4112788 and rs4085613 were significantly associated with increased susceptibility to PsA ($p_{\text{trend}} = 0.001$, OR = 1.19 and $p_{\text{trend}} = 0.001$, OR = 1.18 respectively). No association was observed to rs6701216.

Conclusions: The evidence presented here supports the LCE deletion as a risk factor for PsA in a UK and Irish population. It suggests that this locus is a risk factor within a shared etiological pathway that contributes to psoriatic skin disease in both PsV and PsA.

P09.117 Dissecting the MHC locus in psoriatic arthritis

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Both psoriasis vulgaris and psoriatic arthritis (PsA) show strong association with SNPs in the major histocompatibility complex (MHC) region on chromosome 6q. It has, however, proven difficult to interpret these data because of complex LD at this region. In a GWAS using 572 German PsA patients and 888 population based controls (KORA), association to this locus was confirmed. To clarify whether the associa-

tion is due to one or several loci within the same region, we performed a stepwise logistic regression analysis using rs13191343 (our most significantly associated SNP) as covariate in the first step, and the most strongly associated SNP from this as an additional covariate in the next one, until logistic p-values for all SNPs on chromosome 6q were above 5.0E-6. We were able to identify 5 distinct SNPs which account for most of the observed association. Of these, 4 were replicated in independent study groups of 1,761 European PsA patients and 3,727 control individuals. They are in no or negligible LD with each other, so we conclude that at least four different loci are responsible for the strong association signals for PsA within the MHC region. Additionally, there was no evidence for log-linear interaction between these 4 loci among each other and with TNF, coding for a psoriasis drug target within MHC. Furthermore, only one locus overlaps one of 3 recently identified loci for psoriasis vulgaris, suggesting that the remainder are specific to PsA and that both shared and distinct genetic factors predispose for psoriasis and PsA.

P09.118** A CNV in the Epidermal Differentiation Complex, affecting the LCE3C and LCE3B genes, is a susceptibility factor for psoriatic arthritis in Spanish and Italian populations.

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Objective: Psoriatic arthritis (PsA) is a complex disorder in which environmental and genetic factors are involved. Recently LCE3C_LCE3B_del has been defined as a susceptibility factor for psoriasis and rheumatoid arthritis, making it a good candidate for PsA. Nonetheless, a study performed on German PsA patients did not find association. The aim of this study was to elucidate whether LCE3C_LCE3B_del is associated with PsA in the Spanish and Italian populations.

Methods: We tested for the association between LCE3C_LCE3B_del and a linked ($r^2=0.928$, $D'=0.98$) SNP (rs4112788) with PsA in three independent case-control datasets (Barcelona: 50 cases and 411 controls, Galicia: 178 and 124, and Roma: 424 and 450). The Barcelona and Roma samples were typed for the presence of the LCE3C_LCE3B deletion by direct PCR and for the SNP with a Taqman assay, and the Galician dataset was only tested for the SNP. Association analysis was performed by R SNPAssoc package.

Results: Analysis of the Barcelona dataset showed significant association of the LCE3C-LCE3B_del and rs4112788 with PsA ($p=0.0075$; OR=2.27(1.25-4)) and ($p=0.03$; OR 1.92(1.06-3.44), respectively). The rs4112788 association was confirmed in the Galician dataset ($p=0.004$; OR 2.22 (1.26-3.84)). A large Italian cohort confirmed both associations (LCE3C-LCE3B_del $p=0.006$; OR 1.63(1.15-2.38); rs4112788 $p=0.008$; OR 1.61(1.44-2.32)). Joint analysis for the SNP showed an overall p -value of $p=0.00019$; OR 1.35(1.19-1.59)). Subphenotype analysis showed a stronger association for the oligoarticular versus polyarticular disease (interaction p -value=0.03).

Conclusions: The LCE3C-LCE3B deletion shows association with PsA in the Spanish and Italian populations analyzed, presenting a stronger effect in the oligoarticular cases.

P09.119 Variants in polyunsaturated fatty acids metabolizing enzymes are associated with neurodevelopment in INMA birth cohort

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de Salut de Menorca (*ib-salut*) and Fundació Caubet-CIMERA, Balears, Spain. Long chain polyunsaturated fatty acids (LC-PUFAs) are essential for cognitive development, mainly during first months of life. Although formula milks have been started to be supplemented with PUFAs, some essential PUFAs are uniquely available in human milk. In the present study we have tested direct and indirect effects of genetic variants in *FADS* and *ELOVL* genes on neurodevelopment. Thirty-six tag SNPs were genotyped with the SNPlex technology in 340 Caucasian children from the Menorca INMA (Infancia y Medio Ambiente) birth cohort. After excluding variants with low genotyping quality or deviation from HWE, twenty seven SNPs were analyzed in relation to neurodevelopment measured at age 4 years with McCarthy Scales of Children's Abilities test consisting in 6 subscales. The statistical analysis was performed using a likelihood ratio test from a linear model adjusting for main confounders. We also tested interactions between genetic variants and breastfeeding (83% breastfeeding, 17% formula milk). After Bonferroni correction, a SNP in the *FADS* cluster was associated with the general cognitive and quantitative scales. Two SNPs in *ELOVL* genes were associated with the perceptual-performance (*ELOVL2*) and motor (*ELOVL5*) scales. Nominally associations were also found for SNPs in these genes and the six McCarthy scales. Finally, an interaction between breastfeeding and a SNP in *ELOVL5* was found for general cognition, verbal, quantitative and motor skills. In summary, genetic variants in PUFA enzymes, directly or through an interaction with breastfeeding, seem to have an effect on neurodevelopment. Due to the limited number of individuals analyzed, results should be replicated.

P09.120 Evaluating the influence of multiple genetic polymorphisms on genitourinary and gastrointestinal acute morbidity to radiotherapy

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Genetic predictive markers of radiation morbidity are being sought for stratifying radiotherapy for patients and risk assessment of radiation exposure. To evaluate the association of genetic variants with radiotherapy-induced acute normal tissue toxicity we enrolled 472 unselected prostate cancer patients, treated at the Radiation Oncology Department (Clinical University Hospital of Santiago de Compostela) from 2006-2009, into a prospective epidemiological study. Adverse effects (AE) were documented for 8 weeks using the common toxicity criteria (CTCv3.0). Patients showing CTC grade ≥ 1 for genitourinary (GU) or gastrointestinal (GI) disorders were comprised in the "case" group. The "control" group consisted of patients without acute clinical sensitivity.

After a systematic review, we selected previously associated SNPs as so as validated non-synonymous SNPs in the same genes. Altogether, 17 SNPs in 6 candidate genes were genotyped. The power to detect odds ratios as low as 1.4 for selected SNPs considering an average population minimum allele frequency (MAF) of 12% was 88% for an allelic test. All SNPs were found in HWE in controls.

The TGFB1 rs1800472 allele T was significantly associated with both the risk of developing GU (OR=1.81; CI95%=1.02-3.22; p=0.04) and GI AE (OR=2.08; CI95%=1.05-4.13; p=0.03), although this association did not reach the nominal significance level after Bonferroni correction.

Due to the present study is the first to investigate the role of rs1800472 in the acute adverse normal tissue reactions to radiation, we understand the need of replicating our results in a greater sample in order to convincingly distinguish the proposed effect.

P09.121 New loci associated with blood cell and iron traits in the isolated population of Val Borbera

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Blood cells participate in vital physiological processes as oxygen transport, defense against infections and vessel wall integrity. Their numbers and characteristics are in part determined by genetic mechanisms (Andrews NC, *Nat Genet* 2009). We used the Val Borbera isolated population in the Appenine region of Northern Italy (Traglia et al, *Plos One* 2009) to define genetic variants associated with blood cell traits and iron parameters. Using 370K Illumina array we conducted genome-wide association studies for 15 hematological and 4 iron traits measured in the peripheral blood of 1664 individuals. These traits include hemoglobin, red cell counts and erythrocyte indices, white cell number and types, platelet and iron parameters. Our analyses replicate 23 loci that recent studies have found associated to blood cells (Soranzo et al *Nat Genet* 2009, Ganesh et al *Nat Genet* 2009, Gudbjartsson et al *Nat Genet* 2009, Chambers et al *Nat Genet* 2009) and iron (Benjamin et al *Nat Genet* 2009) traits. Moreover we found 3 new loci that reach the genome-wide significance associated to mean corpuscular hemoglobin concentration (MCHC), neutrophils count and platelet distribution width (PDW). These preliminary results require replication in other populations, sequencing analyses and functional follow-up to prove their true involvement in quantitative traits variation.

P09.122 The RET51/FKBP52 complex and its involvement in Early Onset- Parkinson disease

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RET51 is a tyrosine kinase receptor expressed in distinct families of neurons where it promotes different functions. FKBP52 is an immunophilin with neuroprotective effects on different kind of neurons. We have demonstrated that RET51 activation by both GDNF and NGF triggers the formation of RET51/FKBP52 complex. The substitution of the tyrosine 905 of RET51, a key residue phosphorylated by both GDNF and NGF, disrupts the RET51/FKBP52 complex. NGF and GDNF have a functional role in dopaminergic (DA) neurons where RET51 and FKBP52 are expressed with a yet undefined function. The degeneration of DA neurons is the main feature of Parkinson Disease (PD), which is associated to a complex multifactorial aetiology combining environmental, age-related and genetic factors. To clarify if RET51/FKBP52 complex should exert its function in DA neurons we used an indirect approach by screening the genes encoding for RET51 and FKBP52 in a group of 30 Early Onset (EO)- PD patients. We found a compound heterozygous carrying two mutations in RET and FKBP52 genes. Functional analysis performed in the human cell line HEK293 have shown that the two mutations expressed simultaneously are sufficient to disrupt the RET51/FKBP52 complex, indicating its potential role in EO- PD.

P09.123 Association study of CARD8 c. 30T_A (p.C10X) variant with Rheumatoid Arthritis in Tunisian patients

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Caspase activating and recruitment domain 8 (CARD8) may be a negative regulator of NF- κ B, which plays a key role in inflammation, and also has a regulatory effect on apoptosis. The common variant rs2043211 (c.30T>A) introduces a stop codon (Cys10Ter) at position 10 of the amino acid sequence (p.C10X). CARD8 full-length (CARD8-L) was recently found to be associated with rheumatoid arthritis (RA). The aim of this study was to analyze the frequency of p.C10X in 141 Tunisian patients affected with RA and 191 healthy unrelated controls. DNAs genotyping was carried out with a TaqMan 5' allelic discrimination assay on an ABI 7500 real time PCR machine (assay: C_11708080_1_) and data were analyzed by chi-squared test, Genotype relative risk and Odds Ratio (OR) with 95% confidence interval (CI). Our results showed neither allelic nor genotypic significant association of the CARD8 gene polymorphism with RA (p=0.42; p=0.115, respectively); [OR (95% CI) = 1.14 (0.83-1.58)]. The stratification of RA patients subgroups according to clinical and immunological data re-

vealed significant associations of T/T genotype in HLA-DRB1*10 positive subgroup (n=20) ($p=0.009$). However, no significant differences of rs2043211 polymorphism was found according to the presence of nodules, another autoimmune disease, erosion, anti-cyclic peptides antibodies (ACPA) and rheumatoid factor antibody (RF) ($p>0.05$). Our results suggest that the CARD8 rs2043211 polymorphism was significantly associated with RA susceptibility in patients carrying at least one allele HLA-DRB1*10. Further replication studies with a larger sample size as well as on different populations are needed to confirm this finding.

P09.124 Functional SNPs in *CD244* gene associated with rheumatoid arthritis in a Japanese population

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Rheumatoid arthritis (RA) is well-known as an autoimmune disease and is a chronic inflammatory disorder. 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 as reported. 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 studied whether variants of the SLAM family gene are associated with susceptibility 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 $P=7.45 \times 10^{-8}$). We found a Japanese cohort of SLE that had the similar genotype distribution with RA cohorts. These disease-associated SNPs have been shown to increase their expression using luciferase assays. Furthermore, we indicated that rs6682654 locates on the binding site of USF-1 in *CD244* gene and affect on the regulation of *CD244* expression via USF-1. We supposed that up-regulation of *CD244* by transcription factors including USF-1 affect on RA. Thus, *CD244* is a novel genetic risk factor for RA and may have a role for autoimmunity in RA.

P09.125 Identification of two susceptibility variants for schizophrenia in Bulgarian sample by case-control association study approaches

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Recent development of molecular psychiatry identified a number of candidate genes possibly associated with schizophrenia. However, the studies often result with controversial and non-conclusive outputs. Replication studies might help to unravel this problem. The candidate gene approach requires selection of a small number of candidate markers out of a limited number of loci suggested by preliminary studies. In contrast genome-wide association studies enable high-throughput screening without previous knowledge of disease etiology or pathogenesis.

We employed a two-step case-control association design in order to reveal schizophrenia susceptibility genes in a Bulgarian sample. Initially we performed a candidate gene replication study of 180 SNPs in 59 candidate genes, using 255 Bulgarian patients with schizophrenia and schizoaffective disorder, and 556 Bulgarian healthy controls (by TaqMan® or Invader assay®). In stage two, a genome-wide association study (Illumina Bead Array (550K)) was performed across 188 affected and 376 healthy Bulgarian subjects. Hundred markers with lowest p-values were validated and then followed up in additional set of 99 case and 328 control samples by Invader assay®.

The candidate gene replication study revealed rs6277 (*DRD2*) ($P = 0.0010$, OR = 1.76) as a susceptibility factor for schizophrenia in our sample. The GWAS identified strongest association with an intronic SNP of an involved in the pathway of coordination between the tissue size and shape and the cell-type identity in brain development.

Our findings support two of the most widely considered hypotheses for schizophrenia's etiology: the dopaminergic hypothesis and the maldevelopment of the nervous system hypothesis.

P09.126 Severe schizophrenia in males is associated with the presence of *MTHFR* 677T allele.

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Objective: *MTHFR* gene codes the methylenetetrahydrofolate reductase, one of the important enzyme of folate metabolism, also playing role in DNA methylation - thus influencing important cellular pathways. The *MTHFR* C677T mutation has been associated with elevated homocysteine levels, that can cause different abnormalities of fetal development: neural tube defects, cardiac dysfunctions and others. Some data points the role of this gene mutation in schizophrenia development.

We studied functional *MTHFR* C677T alleles variation within schizophrenic patients families and in control group in order to check if any association exists between carriers of 677T allele and disease.

Method: 116 schizophrenic patients as well as their mothers and 57 healthy control families were included in research. The patients were differentiated into 2 disease severity groups according clinical data. Allelic frequencies for the C677T polymorphism were estimated for all studied persons.

Results: Strong 677C/T allele ratio frequencies shift was revealed in the group of male patients with mild defect (85/15) as compared to severe defect group (66/33) and controls (68/31). We didn't observe any shift in 677C/T frequencies for female patients. Significant differences ($P=0.001$) were discovered between groups of male and female patients, subdivided by the severity of disease (mild and severe defect), in ratio of individuals bearing 677T allele. Male patients with 677T allele more often suffer from severe schizophrenia.

Conclusions: To our mind, obtained data reflect the different way of schizophrenia development in male and female patients with mild and severe schizophrenia.

P09.127 Meta-analysis of 4 European genome wide association scans identifies 3 new QTLs influencing serum amyloid A concentrations

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Serum amyloid A (SAA), a sensitive marker of an acute inflammatory state, is associated with several severe chronic diseases, such as

amyloidosis, Alzheimer's disease, tumor genesis and rheumatic arthritis. Furthermore, SAA concentrations have been reported to be linked to obesity, atherosclerosis, and diabetes, three of the risk factors for cardiovascular disease. We conducted a meta-analysis of 4 genome-wide association scans on baseline SAA concentrations totalling 4,264 participants of European descent (KORA S4, LURIC, Sorbs, TwinsUK) and identified 3 independent signals which are distributed across two regions. One region is located on chromosome 11 and displays two independent loci, one of which harbouring SAA1 and SAA2 and the other the neighbouring GTF2H1 and HPS5. The other region includes the LEPR gene region on chromosome 1. Taken together, the present meta-analysis was the first genome wide association analysis on SAA concentrations and detected gene regions, which highlight immune response pathways involved in the regulation of chronic inflammation and implicated diseases and underline a close interplay between SAA and other inflammatory proteins.

P09.128 Synaptic exocytosis and migraine: association study of the SNARE complex and related genes in a Spanish population

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Migraine is a complex multifactorial neurological disorder. It has been suggested that disturbances of ions and/or neurotransmitter levels in the synaptic cleft may be involved in the pathophysiology of migraine by influencing neuronal excitability. The Soluble N-ethylmaleimide sensitive factor Attachment protein REceptor (SNARE) complex plays a key role in the fusion of vesicles loaded with neurotransmitter with the presynaptic membrane. The aim of the present study is to investigate the involvement of 15 genes (STX1A, SNAP25, VAMP1, VAMP2, SYT1, SYT2, CPLX1, CPLX2, CPLX3, CPLX4, STXBP1, SYP, SNPH, NSF, NAPA) encoding proteins that belong to or interact with the SNARE complex through a case-control association study of 538 migraine patients, 312 without aura (MO) and 226 with aura (MA), and 538 sex-matched controls from Spain. The analysis of 144 SNPs displayed several nominal associations in different clinical groups, but only two SNPs in CPLX2 (complexin 2) for MO and one SNP in NSF (N-ethylmaleimide-sensitive factor) for MA remained significant after 15% FDR correction for multiple testing. A replication study with 480 cases and 480 controls from Spain is currently underway.

P09.129 Replication of the Celiac Disease GWAs results in Spanish population

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BACKGROUND: Celiac disease (CD) is a chronic, immune-mediated disorder of the gut caused by intolerance to ingested gluten that develops in genetically susceptible individuals, affecting approximately 1% of Caucasians. The major genetic risk factors for CD are HLA-DQ2/DQ8 molecules. Nevertheless, these variants are also frequent in the general population, so HLA is necessary but not sufficient to explain all genetic susceptibility to CD. Genome-wide association studies (GWAS) have been performed in CD and eight new loci that contribute to genetic risk have been identified and replicated in different populations.

AIM: Our aim was to replicate the association of the eight risk loci in the Spanish population.

PATIENTS AND METHODS: We genotyped 500 CD patients and 470 healthy controls from the Spanish Celiac Disease Genetics Consortium collaborative study for GWAs associated single nucleotide polymorphisms (SNPs) tagging the eight risk loci. SNPs were genotyped

using TaqMan probes and primers on an ABI Prism 7900HT instrument. SDS v2.3 software was used for genotype calling. Association analyses were performed using 2x2 contingency tables.

RESULTS AND CONCLUSIONS: Significant association was observed in five regions (Table 1), confirming the implication of several those genomic regions in CD susceptibility in a southern European population.

| LOCUS | SNP | Minor allele | MAF cases/controls | p Value |
|--------------|------------|--------------|--------------------|---------|
| RGS1 | rs2816316 | C | 0.145/0.199 | 0.0002 |
| IL12A/SCHIP1 | rs17810546 | G | 0.132/0.100 | 0.0286 |
| IL12A | rs9811792 | C | 0.514/0.439 | 0.0003 |
| SH2B3 | rs3184504 | T | 0.510/0.438 | 0.0004 |
| KIAA1109 | rs13119723 | G | 0.178/0.229 | 0.0024 |

The majority of GWAs identified loci are replicated across populations, although allele frequencies may vary. Failure to replicate may be in part due to differences in genetic background.

P09.130 The I/D polymorphism of the ACE1 is not a risk factor but is a prognostic marker for ischemic stroke

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Background: Studies on the angiotensin-converting enzyme (ACE1) gene in ischemic stroke (IS) have generated conflicting results. The goal of our study was to clarify the influence of the ACE1 as a risk factor and prognosis marker for IS.

Methods: We genotyped the rs4341 polymorphism, in linkage disequilibrium with the rs1799752 (ID polymorphism) in 531 IS cases and 549 healthy controls. We then tested if the rs4341 could influence thrombolytic efficacy (measured as frequency of revascularization) and safety (measured as cerebral hemorrhagic complications) in IS patients who received t-PA <3h after symptoms onset. We measured serum ACE levels in 130 samples: 27 controls, 68 IS at baseline and 35 IS 24h after stroke.

Results: There was no association of the ACE1 variant with IS ($p=0.776$), although it affected ACE protein levels ($DD=153.5\pm54.9$, $ID=120.4\pm39.8$, $II=107.0\pm45.8$ ng/mL, $p<0.001$). IS cases showed lower ACE levels than controls in the acute phase ($p<0.001$), but not at 24h ($p=0.673$). On another hand, the D allele was associated with higher revascularization rates in tPA-treated patients ($p=0.037$ at 1h), without increasing the odds of hemorrhagic complications ($p=0.244$). Protein levels at 24h were also higher in IS patients who revascularized at 24h (174.6 ± 47.9 vs. 116.0 ± 28.1 , $p=0.028$).

Conclusion: The D allele of the ACE1 I/D and ACE protein levels were not associated with a higher risk of IS in Spanish individuals. However, the ACE1 could be a good pharmacogenetic marker, since it is associated with higher revascularization rates following t-PA treatment.

P09.131 Correlation between different clinical manifestations and subphenotype stratification of genetic association in Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is a complex genetic disease with heterogeneous clinical manifestations. An individual can be classified as having SLE when 4 or more of the 11 criteria set by the American College of Rheumatology (ACR) have been met. Disease manifestations are therefore varied and diverse, where two SLE patients may have non-overlapping subphenotypes. It is known that multiple genes are involved in SLE. The phenotypic heterogeneity could be a result of an underlying genetic heterogeneity, with different subsets of susceptibility genes contributing to different phenotypic manifestations. In this study, we analysed the clinical data from 1211 SLE patients collected in Hong Kong, including the 11 criteria from the ACR classification criteria and autoantibody production. We also correlated this with the genotype data for 612 of these patients genotyped by the Illumina 610-Quad Beadchip in a recent genome-wide association study and the genotype data of several well-established SLE susceptibility genes for the remaining patients by the Sequenom MassARRAY iPLEX Gold system. Correlation analysis between disease manifestations revealed

that several subphenotypes (clinical manifestations and autoantibody production) tended to cluster together, indicating overlapping of the underlying mechanisms for these manifestations. Furthermore, patient-only analysis (e.g. patients with arthritis versus patients without) showed that many well-established susceptibility genes may be particularly associated with specific disease subphenotypes. Our study may help dissect the intrinsic correlation between different subphenotypes and the correlation between genetic makeup and clinical manifestations.

P09.132 Dissection of genes in the type I interferon pathway reveals two novel risk genes for SLE

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Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease in which the type I interferon (IFN) pathway plays a crucial role. We have previously shown that five genes in this pathway, *IRF5*, *TYK2*, *STAT4*, *IFIH1* and *IRF8* are associated with risk for SLE, as well as successfully replicated the previous reports on association for *IRAK1*, *TNFAIP3*, *TNFSF4* and the *IRF7* region (KIAA1542). Here we investigate 78 genes involved in the type I IFN pathway to identify additional SLE susceptibility loci. First, we genotyped SNPs in these 78 genes and 14 other candidate genes in Swedish SLE patients and controls. Genes with $P < 0.01$ in the initial screen were then followed up in an additional Swedish cohort. SNPs in five genes were nominally associated with SLE in this extended cohort. To replicate these findings we extracted data from a genome-wide association study on SLE performed in a US cohort. Combined analysis of the Swedish and US data confirmed two of these genes as SLE susceptibility loci. Our study highlights additional genes from the type I IFN system for further functional analysis, and more specifically points to the importance of genes in the *IFIH1/DDX58* pathway which is activated in cells other than the plasmacytoid dendritic cells, for example monocyte derived dendritic cells, in response to viral infections.

P09.133 BK channel $\beta 4$ subunit gene (KCNMB4) and Temporal Lobe Epilepsy

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Epilepsy is one of the major neurological disorders characterized by spontaneous and recurrent seizures. Traditionally Temporal Lobe Epilepsy (TLE) was considered as a multifactorial syndrome due to environmental factors. Advances in molecular biology have facilitated the detection of many genetic alterations that may have a pathogenic effect in TLE. Large conductance calcium-activated potassium channels (BK) have recently been implicated in the pathogenesis of genetic epilepsy.

The objective of this study is to replicate previously published results regarding the involvement of KCNMB4 gene in TLE. We used a case-control approach comparing a tag single-nucleotide polymorphism (SNP) located 3' of this candidate gene between unrelated TLE patients and matched controls. A total of 359 patients and 319 age- and

sex-matched healthy controls were genotyped for a single biallelic (G/T) nucleotide polymorphism (rs398702) in the 3' region of the KCNMB4 gene using a TaqMan 5' allele discrimination assay. Analysis of genotype or allelic frequencies between patients and controls showed no statistically significant difference ($p > 0.05$). Our data suggest that KCNMB4 gene polymorphism does not act as a susceptibility factor for TLE. Although these findings do not replicate the earlier findings at the SNP level, we believe that many SNPs contribute to disease predisposition in an population-specific manner. Despite our negative results, the candidate gene selection strategy could be quite useful in the future determination of variants predisposing to disease.

P09.134 Genotyping of the TPMT gene using pyrosequencing in Polish population

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Thiopurine methyltransferase (TPMT) metabolizes thiopurine drugs which are used in the treatment of several disorders such as inflammatory bowel disease (IBD). The therapeutic function of thiopurine drugs is considerably dependent on the genetic polymorphisms of TPMT gene. The three main TPMT alleles, TPMT *2 (p.Ala80Pro), *3A (p.Ala154Thr, p.Tyr240Cys) and *3C (p.Tyr240Cys), determine 80 - 95% of the intermediate and low enzyme activity, and increase the risk for thiopurine-induced toxicity. The aim of our study was to optimize a simple genotyping method to identify main TPMT alleles and to determine the frequency of these changes in the Polish population. We tested 37 of IBD patients treated with thiopurine drugs and 100 controls. Following a PCR protocol, fragments of exon 4, 6 and 9 of TPMT gene enclosing codon 80, 154 and 240, respectively, were analyzed using pyrosequencing. The results were compared with those obtained by standard sequencing. Identification of these sequence variants using pyrosequencing is highly sensitive and less time consuming compared to standard sequencing or restriction fragment length polymorphism (RFLP). It can be easily integrated in diagnostic testing. Moreover, we found a TPMT*3A allele frequency of 8,1 % in IBD patients compared to 3,0 % among general Polish population. The frequency of TPMT*3C allele in heterozygote is of 2,7% in IBD patients and 0,0% in controls. TPMT*2 allele was not identified either in patients or the Polish control group.

P09.135 Quality control for large-scale high throughput mRNA isolation and transcriptome analyses of subjects of the Rotterdam Study

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Introduction: Within the Rotterdam Study (RS), Genome-Wide Association Studies have successfully identified genomic regions associated with several diseases and traits. To identify the genes involved we will study genotype-dependent expression profiles of blood mRNA in 2,000 subjects, but first evaluated quality of transcriptomes of 96 RS participants.

Materials & Methods: Whole blood cells have been collected for ~2,000 RS subjects. RNAs were isolated (Qiagen PAXgene-tubes), amplified and labelled (Ambion Illumina TotalPrep), and hybridized to the Illumina Whole-Genome Expression Beadchips (HT-12). For normalization and analysis, the *lumi* package of Bioconductor was used (Du *et al.*, 2007).

Results: For the pilot of 96 RS subjects 90.6% had sufficient quality ($RIN > 7.0$) and RNA yield ($> 1.0 \mu\text{g}$). After amplification, 98.9% had a cRNA yield $> 5.0 \mu\text{g}$. A total of 70 samples were hybridized to 6 Illumina WG Expression Beadchips, containing 48,775 probes. After variance-stabilizing transformation and quantile-normalization, 3 samples (low intensities) and 25,386 unexpressed probes ($p < 0.01$) were excluded. The remaining 67 samples did not cluster by beadchip, date of blood collection, the person who collected the blood, nor the person who isolated the RNAs. Age and gender were not found to influence the expression profiles.

Discussion: In this pilot set-up we detected no biases in transcriptomes during sample collection, preparation or hybridization, consequently

transcriptome analyses of further samples is warranted. On the long run, we will try to replicate earlier reported expression quantitative trait loci (eQTLs) from lymphocytes, while for 4 RNAs microarray-data will be compared to RNA-seq transcriptome-data (generated with Illumina-GAI).

P09.136 TRP channels: a case-control association study with migraine

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Migraine is a common episodic, chronic and disabling cephalgia with a complex inheritance that is caused by the interaction among multiple genes and environmental factors. The fact that rare monogenic forms of the disorder are caused by mutations in genes involved in ion transport point at these genes as potential candidates to underlie also the common forms of migraine. Transient Receptor Potential (TRP) channels constitute a heterogeneous family of cationic non-selective channels that work as cellular sensors, playing critical roles in a number of physiological processes such as touch, vision, olfaction or thermo- and osmosensation. TRP channels have been involved in several neurological disorders including Charcot-Marie-Tooth type 2C, Guamanian amyotrophic lateral sclerosis and mucolipidosis type IV. We have studied the possible contribution of 14 genes of the TRP superfamily (*TRPA1*, *TRPC1*, *TRPC3*, *TRPC4*, *TRPC5*, *TRPC7*, *TRPM4*, *TRPM6*, *TRPM7*, *TRPM8*, *TRPV1*, *TRPV2*, *TRPV3*, *TRPV4*) to migraine through a case-control association study with 192 single nucleotide polymorphisms (SNPs). We genotyped 555 migraine patients (124 males and 431 females, 323 migraine with aura -MA- and 232 migraine without aura -MO-) and 555 controls. Nominal associations were identified with 11 of these genes, but none of them overcame multiple testing corrections. However, we identified a risk haplotype for migraine in the *TRPC4* gene, while *TRPC1*, *TRPM6* and *TRPM8* contained susceptibility haplotypes to MO and *TRPM4*, *TRPV4*, *TRPV3* and *TRPV1* to MA. These results await replication in a second Spanish case-control sample, currently underway.

P09.137 The study of association between polymorphism of genes responsible for Th1/Th2-polarization and tuberculosis in Russian population of Siberia

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The analysis of inherited basis of predisposition to tuberculosis (TB) is one of the most perspective and actively-developing field of genetics of infectious disease in humans. More than twenty candidate genes underlying the predisposition to TB were discovered; however, the effects of these genes vary in different populations. The aim of this study was to search for an association of SNP markers of genes responsible for Th1/Th2-polarization with TB. The study was performed in a group of 304 Russians from Siberia (Tomsk Region, Russia) with TB and 160 healthy controls. We investigated genes encoding for cytokine ligands and receptors (*IL12B*, *IL12RB1*, *IFNG*, *IFNGR2*, *IL4*, *IL4RA*, *IL1B*, *IL1RN*) and genes involved in intracellular transduction of immune signals (STAT-, SOCS-, PIAS-family genes). The following associations between polymorphic markers and TB were observed: *IL12B* (rs3212227; p=0.044), *PIASY* (rs760903; p=0.019), *PIAS3* (rs12756687; p=0.019). The SNPs in *IL12B* and *PIASY* were mainly associated with secondary TB (p=0.041 and p=0.027, respectively), whereas the polymorphism of *PIAS3* was associated with primary TB (p=0.001). So, the genes of PIAS-family are new candidate genes of predisposition to TB. This observation can reflect the fact that the genes studied influence a balance between Th1/Th2-polarizing sig-

nals and, therefore, determine predisposition to different clinical forms of TB. This hypothesis requires further confirmation.

P09.138 Validation of a novel chromosome 10p CNV associated with Type 2 diabetes

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The use of SNP genotyping data to identify copy number variants (CNVs) is potentially a highly effective approach for the detection of novel common and rare genetic variants associated with disease. In order to identify common CNVs associated with Type 2 diabetes (T2D), we applied our novel algorithm cnvHap to Illumina genotyping data from stage 1 of a GWAS in a French cohort, for approximately 300,000 SNPs from 694 cases and 669 normoglycaemic controls. We have characterised in detail a duplication located on chromosome 10p, encompassing a SNP for which increased copy number is associated with an increased risk of T2D (p value <10⁻¹⁰). The variation in copy number observed at this locus is caused by a combination of a small, 70bp duplication and a larger duplication involving all or part of a 20.8kb repeat block. Genotyping of the 70bp duplication reveals that, by itself, this variant does not explain the CN association of this SNP with T2D: the allele frequencies are 0.076 in normoglycaemic controls (n=909) and 0.074 in cases (n=890). Thus, it appears that the large duplication is responsible for the observed CN association at this locus, either solely or in combination with the 70bp duplication. Ongoing qPCR measurements to genotype the large duplication will enable confirmation of its association with T2D and interrogation of any possible interactions between the copy number state of the two duplications at this locus.

P09.139 Hsp70 genes polymorphisms: lack of association with Undifferentiated Spondyloarthritis in Romania

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Background: Undifferentiated spondyloarthritis (USpA) belongs to the group of spondyloarthritides, rheumatic inflammatory diseases caused by genetic, environmental and immunologic factors.

Heat shock proteins (hsp) genes polymorphisms have been investigated in different conditions with an immune component, including spondyloarthritides. The +1267A/G polymorphism in hsp70-2 gene showed a significant association with USpA in Mexican Mestizo patients.

Objectives. The aim of this study was to investigate the association of two known polymorphisms (+190G/C and +1267A/G of the hsp70-1 and hsp70-2 genes) with undifferentiated spondyloarthritis in Romania.

Methods. 75 unrelated Romanian patients with USpA (diagnosed according to the European Spondyloarthropathy Study Group criteria) and 100 healthy unrelated ethnically matched controls were involved in this study. Patients did not meet criteria for any of the well defined spondyloarthritides.

All subjects were genotyped for hsp70-1 +190 G/C (rs1043618) by TaqMan SNP Genotyping Assay C_11917510_10 (Applied Biosystems, USA) and for hsp70-2 +1267A/G (rs1061581) by PCR-RFLP with PstI restriction enzyme. Association tests for each polymorphism and haplotype frequency estimations were performed with software package PLINK v 1.07.

Results. Patients and controls groups were in Hardy-Weinberg equilibrium for both polymorphisms. No association was found between any of the investigated polymorphisms and USpA. Three main haplotypes were constructed, the most common being 190G/1267A (65% in patients and 62% in controls). There was no association of these haplotypes with the disease.

Conclusion. The present study shows no association of hsp70-1 +190G/C and hsp70-2 +1267A/G polymorphisms and derived haplotypes with susceptibility to undifferentiated spondyloarthritis in Romanian patients.

P09.140 Mthfr gene polymorphism c677t associated with urinary tract anomalies in girls

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Background: Folate deficiency could play a role not only in neural tube defects (NTD), but also in urinary tract anomalies (UTA) formation. We evaluated the frequency of methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms C677T and A1298C in patients with UTA and NTD, comparing them to healthy Slovak children.

Methods: The cohort comprised 515 children: 225 patients (132 UTA /85 boys/; 93 NTD /39 boys/ and 290 healthy newborns (147 boys). The patients' DNA was analysed by PCR followed by enzyme restriction. The newborns' DNA isolated from dry blood spot was analysed by RT PCR. Genotype frequencies were compared with Pearson χ^2 or Fisher tests. Odds ratio was rated by Garts method, statistic significance level was determined as ≤ 0.05 .

Results: The Slovak population frequencies of T allele and TT genotype of C677T polymorphism were 25,17% and 6,90%; C allele and CC genotype of A1298C: 34,66% and 13,79%; no differences between the sexes. NTD patients: no association with MTHFR polymorphisms, no sex differences. Surprisingly, UTA patients showed significantly higher incidence of C677T polymorphism compared to the controls (T allele resp. TT genotype: 32,95%, $p=0.019$ (OR=1,9957; 95%CI[1,0086-3,9486]) resp.12,88% $p=0.044$ (OR=1,4611 95%CI[1,0633-2,0078]). This finding was even more significant in girls with UTA: T allele resp. TT genotype frequencies 42,55%, $p<0.0001$ (OR 95%CI 2,676 [1,629-4,396]) resp. 19,20% $p=0.008$ (OR; 95%CI 3,9967:[1,2615-12,6931]) compared to the healthy girls. On the other hand, T allele in UTA boys represented 27,6%, compared to 42,6% in girls, $p=0.0136$. A1298C polymorphism in UTA group did not differ from the controls. Conclusions: Despite no association of MTHFR gene polymorphisms with NTD in our patients, MTHFR gene polymorphism C677T was strongly associated with UT anomalies in girls. This might point to UT developmental sex-differences related to sex-specific processes of methylation, which might be limited due to reduced MTHFR activity. This hypothesis should be further tested on larger groups of patients.

P09.141 The pattern of genetic inheritance of voice registers

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Little is known about the genetics of voice. Vocal registers arise from several anatomical configurations which lead to different vibratory patterns produced by the vocal cords. The purpose of this retrospective study was to analyze the different vocal registers in 55 men (tenor, baritone and bass) and 45 women (soprano, mezzo-soprano and contralto), their descendants and ascending relatives, and to suggest a pattern of inheritance for vocal registers. These people were lyric singers of Teatro Municipal, and of two Schools of Music in Rio de Janeiro (UFRJ and UNIRIO). The voice registers, when unknown, were determined by the lyric singer Maria Bezzi. In this work we observed the limited variability of the vocal registers and timbres within families, and found a high frequency of tenors (30%) and sopranos (28%) in the sample. We also found mezzo-sopranos (14%), baritones (11%), contraltos (3%), and basses (14%). Forty-five men out of 55 and 41 women out of 45 knew their parents' voice registers. Pedigree analysis showed a high similarity of timbres among men and women within the same family. We suggest a non-mendelian, polygenic and multifactorial inheritance. Each register and timbre has a wide "norm of reaction". Therefore, an enormous complexity can exist in the interrelationships between genetic and environmental factors in determining traits such as voice registers and timbres. Additional genetic research is needed to further elucidate the relationship between vocal tract structure and function, mechanisms of voice and transmission of voice characteristics, and this work is one relevant initial step.

P09.142 Polymorphisms in WNT family genes are associated with nonsyndromic cleft lip and palate

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Nonsyndromic cleft lip with or without cleft palate (NSCLP) is a common birth defect with a prevalence of 1/700 live birth worldwide. NSCLP is a complex trait most probably caused by multiple genetic loci interacting with environmental factors and other genes. Genetic variations in several genes have been identified as contributing to NSCLP up to 12-25%. Vast majority of the genetic susceptibility loci have not been defined and study results are conflicting.

The *WNT* (wingless-type MMTV integration site family) (MIM:165330/602864) family genes are involved in regulation of mid-face development and upper lip fusion; therefore they are strong candidates for an etiological role in NSCLP.

The aim of the study was to confirm *WNT3* and *WNT9B* genes etiological role in the development of NSCLP.

Materials and methods: 108 nonsyndromic cleft lip (CL) and cleft lip with cleft palate (CLP) cases and 182 healthy, unrelated and randomly selected individuals as controls from Latvia were genotyped for 29 SNPs by APEX technology, developed by Tartu University, Estonia (A. Metspalu).

Association analyses of case-controls were applied by PLINK software, after data genetic cleaning.

Results All SNPs were in Hardy-Weinberg equilibrium. Three SNPs out of 29 (rs11655598, $p<0.001$; rs11653738, $p=0.001$; rs4968282, $p=0.021$) showed a significant association with nonsyndromic CLP.

Conclusion: Our data confirms *WNT* family genes role in the development of nonsyndromic CLP. It was considered *WNTs* interacts with *FGFs* and *BMPs* during embryonic development and failure in this pathway leads to formation of NSCLP.

J09.01 Association between the Polymorphism of *SORL1* and Alzheimer's disease

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The genetic epidemiology of late-onset Alzheimer's disease (LOAD) remains a very active area of research, making it one of the most prolifically published areas in medicine and biology. Numerous putative candidate genes have been proposed. A recent study reported significant association of late-onset Alzheimer's disease (LOAD) with multiple single nucleotide polymorphisms (SNPs) and haplotypes in *SORL1*, a neuronal sortilin-related receptor protein, known to be involved in the trafficking and processing of amyloid precursor protein. We examined the co-segregation of six statistically significant single-nucleotide polymorphisms with the *SORL1* gene, in a total of 150 patient samples, and 150 healthy controls from west northern Iran (Eastern Azerbaijan). The samples were genotyped for the polymorphisms and the genotype frequencies were statistically analyzed. One of the SNPs was diagnosed to be individually linked to the gene of study, while the others were linked as haplotypes.

J09.02 No major genes in autoimmune thyroid diseases: Complex segregation and epidemiological studies in a large pedigree from south of Tunisia

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Objective: The objective of this study is to determine epidemiological parameters and transmission mode of autoimmune thyroid diseases (AITDs) in a Tunisian district which harbour a unique multigenerational family with high prevalence of AITDs (Akr). STUDY DESIGN AND Setting: 113 AITDs patients have been subjected to a regular clinical follow-up for over 8 years (1992-2000). The coefficient of consanguinity, incidence and prevalence were determined. On the other hand, a complex segregation analysis was performed in order to determine AITDs mode of inheritance. Results: We have assessed prevalence (43.6%)

and incidence (7.2 per 1000 inhabitants) of AITDs in this district. The mean of consanguinity was estimated at 3% in Akr family and 2.1% in control group. Complex segregation analysis gave evidence for a polygenic character of these diseases when compared to the full model (χ^2 (ddf=6)= 68.31; $p<10^{-12}$). The plausible hypothesis of transmission was the co dominant Mendelian inheritance (χ^2 (ddf=3)=60.89; $p<10^{-12}$). CONCLUSION: Such sample was of a particular utility to foresee transmission mode of AITDs genetic component. The high incidence of AITDs in such a district could be explained by the high rate of endogamy and consanguinity. A larger scale study should be performed in order to establish epidemiological parameters of AITDs in Tunisia.

J09.03 ADRB2 and GNB3 gene polymorphisms at children with bronchial asthma.

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Background: β_2 -adrenergic receptor (ADRB2) connected with Gi-protein may contribute the development of allergic diseases and play role in bronchial asthma (BA) treatment influencing the response on β_2 -agonists.

Aim: To study combination of polymorphisms Arg16Gly (R>G), Gln-27Glu (Q>E) of ADRB2 gene and C825T polymorphism in the gene encoding the G protein β_3 -subunit (GNB3) in boys and girls with BA and healthy children.

Methods: We included 273 Caucasian children; aged of 4-17 years; 227 boys (82,6%) and 46 girls (17,4%) affected by BA. Control group included 148 healthy children; 77 boys (52,1%) and 71 girls (47,9%) aged of 4-17 years. The genetic polymorphism of ADRB2 and GNB3 genes was performed by PCR-RFLP standard method.

Results: In group of children with BA the combination of genotypes RR/QQ/CT prevails comparing to healthy children (7,7% and 1,4%; $\delta=0,006$; OR=5,36; 95%CI 1,28-22,55). The combination of genotypes GG/QE/CT was rarely in children with BA comparing to healthy children (5,5% and 14,0% accordingly, $\delta=0,006$; OR=2,61; 95%CI 1,37-4,94).

Conclusions: Our results show that combination of genotypes and alleles ADRB2 and GNB3 are different in children with BA and healthy children.

Identification of genetic combination of "candidate" genes for BA may be useful for prediction of disease and patient's response to treatment.

J09.04 Association of the CYP2J2*G50T promoter polymorphism with skin allergy in females

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Cytochrome P450 enzyme, CYP2J2, metabolizes arachidonic acid to epoxyeicosatrienoic acids (EETs). These eicosanoids exert anti-inflammatory, vasodilator, anti-thrombotic and antioxidant affects. The G50T promoter polymorphism in the CYP2J2 gene was found to be associated with an increased risk of coronary artery disease (Spiecker, 2004; Borgel, 2008) and bronchial asthma (Polonikov, 2007). In the present study we investigated the association of the CYP2J2*G50T polymorphism with the risk of allergic disorders in patients from Republic Bashkortostan (Russia). A total of 468 subjects were recruited into this study, including 116 patients with allergic asthma, 58 with non-allergic asthma, 70 patients with allergic rhinitis, 64 with allergic skin disorders (contact dermatitis, hives, eczema) and 160 controls. The frequencies of the CYP2J2*G50T genotypes did not differ significantly between patients with allergic, nonallergic asthma, allergic rhinitis and controls. The frequencies of the CYP2J2*G*T genotypes were 6,9% in patients with allergic asthma, 5,2% nonallergic asthma, 7,6% - allergic rhinitis and 9,5% in controls. The frequencies of the CYP2J2*T*T genotypes were 0,9%, 0%, 1,5% and 0,9%, respectively. Women with skin allergy had a higher prevalence of the CYP2J2*G*T genotype (17,6%) than the gender-matched control group (6,3%, $\chi^2=4,92$; $p=0,027$; OR=1,32). No association was observed for skin allergy in males. Thus, CYP2J2*G50T polymorphism may contribute to the pathogenesis of skin allergy in females. Further research is needed to obtain more comprehensive results.

J09.05 Contribution of the MHC class III region to the predisposing role of MHC class II haplotypes in type 1 diabetes

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Relationship between the human MHC region and type 1 diabetes (T1D) has been widely investigated and susceptibility to the disease has been linked to class II alleles. Recent data indicate that other genes located beyond the class II region may have modifying effect on genetic predisposition to T1D, however, the high frequency of the extended ancestral haplotypes in this region hinders the precise localization of the gene variants which are responsible for the observed increased risk.

In order to evaluate the role of genes in class III and class I regions, we collected 42 Hungarian families with at least one affected offspring. Beside HLA-typing, genotype of five polymorphisms and copy number of C4 genes located in the MHC III region was determined. Statistical analysis was performed applying transmission disequilibrium test and chi-square test in a case-control design. As control, 48 Hungarian families with no T1D child were used.

Our data clearly established the previously known class II risk haplotypes, and furthermore we found that the odds ratio of the class II risk alleles, e.g. DQ8-DR4 differed according to the linked MHC III haplotypes. Our results did not show any influence of the different alleles located at the MHC I region.

In conclusion alleles in the MHC class III region may have modifying role on the major susceptibility determinants of T1D located in the class II region. These results may contribute to a more precise determination of individual risk to T1D and may allow the identification of the responsible gene variants.

J09.06 Association of eNOS rs2070744 polymorphism with diabetic nephropathy in diabetes type II patients.

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Diabetic Nephropathy (DN) due to type II diabetes is the leading cause of End Stage Renal Failure. Epidemiological data suggest that there is heterogeneity among type II diabetes patients in different populations, which may be partly explained by genetic predisposition to the development of renal and cardiovascular complications of diabetes. However, the molecular mechanisms have yet to be elucidated. Potential candidate genes include the RAAS, and the endothelial nitric oxide synthase (eNOS) genes. The eNOS is involved in several processes and its dysfunction may lead to diabetes complications, including DN. We assessed the association of eNOS polymorphisms T-786C, Glu-298Asp and 4a/b with type II DN patients. Study subjects comprise of 40 normoalbuminuric diabetes type II (T2DM) patients with duration over 5 years and without antihypertensive treatment and 30 T2DM patients with DN. Genotyping was performed by Real Time PCR melting curve analysis and/or PCR-RFLP.

The frequencies of the eNOS genotypes CC, TC and TT were 0.2, 0.3 and 0.5 respectively, in the control group and 0.17, 0.6 and 0.23 respectively in the case group. Our data showed that the combined frequencies of the eNOS TC and CC genotypes were significantly higher in T2DM patients with DN (0.77) compared to T2DM patients without DN (0.5) ($P = 0.0362$ using Fisher's Exact Test, 95%CI = 1.037-2.163). No statistically significant differences were observed for Glu298Asp and 4a/b polymorphisms or the mutant haplotype -786C/298Asp between the two groups.

Our results suggest a potential association between the mutant -786C allele and DN among T2DM patients.

J09.07 Risk genotypes in SCN5A and ANK2 genes for hypertrophy in patients with severe arrhythmias

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Purpose: The aim of the study was to evaluate possible association of two polymorphisms in long QT genes (C5457T in SCN5A gene and T3579C in ANK2 gene) with hypertrophy in patients with implantable cardiovertor defibrillator after severe arrhythmia episodes.

Methods: Genotypes of the polymorphisms were determined in a sample of 33 Czech patients, aged 62 ± 8 years with implantable cardiovertor defibrillator for severe arrhythmias (85% patients with ventricle fibrillation, 15% with ventricle flutter, EF = 35 ± 12 %, with hypertrophy confirmed in 48 % of patients).

Results: We observed a significantly lower occurrence of hypertrophy in patients with CT genotype of T3579C polymorphism in ANK2 gene compared to CC+TT genotypes of the polymorphism (odds ratio=0.56, P=0.02, with sensitivity of 30%, specificity of 60%). The CT genotype of C5457T polymorphism in SCN5A was associated with higher risk of hypertrophy in comparison with CC genotype (odds ratio = 4.25, P=0.04; sensitivity=33%, specificity 100%). A significant difference in interventricular septum diameter between these two genotypes in SCN5A gene was proved (P=0.005). When both polymorphisms were tested simultaneously, a significant risk for all other associated genotype combinations compared to CT(SCN5A)CT+CC(ANK2) genotypes was found (OR=6.19, P=0.02, sensitivity =65%, specificity = 77%).

Conclusions: In patients with severe arrhythmias with necessity of implantable cardiovertor defibrillator, interactions of long QT genes variability can be expected. Some combinations of these genotypes can be related to hypertrophy in these patients.

J09.08 MTHFR, F2 and F5 genotyping in hemodialysis patients in Bosnia and Herzegovina

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MTHFR (methylenetetrahydrofolate reductase) thermolabile polymorphism (677C-T mutation) is associated with several diseases such as homocysteineuria, thrombosis, and, among others, renal function. F2 (coagulation factor II, prothrombin) 20210G-A gene mutation is associated with an increased risk of venous thrombosis, as well as F5 (coagulation factor V Leiden) 1691G-A gene mutation.

In this study, we genotyped 40 patients with total renal failure which currently undergo hemodialysis. For materials we used 6 ml of whole blood for DNA isolation. Samples were genotyped for MTHFR 667C-T, F2 20210G-A and F5 1691G-A mutation by RFLP method with HindIII and HinfI enzyme, respectively.

Regarding MTHFR genotypes in our sample we found 45% of CC - wild-type genotype, 37.5% of CT genotype and 17.5% of TT genotype. Allele frequencies for C and T allele are 0.6375 and 0.3625, respectively. In our sample we found only one heterozygote (GA) for F2 20210G-A and F5 1691G-A mutation. No mutant homozygote (AA) was found. Allele frequency for G allele in both F2 and F5 gene was 0.9875 and for A allele was only 0.0125. After genotyping, we performed chi-square test on our frequencies compared to NCBI SNP database, and found no statistical significance between our population and world population in allele frequencies (MTHFR - p=0.105; F2 - p=0.886; F5 - p=0.841).

The most common haplotype (MTHFR, F2, F5) for these three genes in our sample was wildtype haplotype - CCAGGG (45%), followed with CTAGGG (32.5%) and TTAGGG (17.5%). Rare haplotypes in our sample were CTAGGA (0.25%) and CTGAGG (0.25%).

J09.09 Angiotensin-converting enzyme activity and polymorphism A1166C of the angiotensin II type 1 receptor at hemorrhagic fever with renal syndrome

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Hemorrhagic fever with renal syndrome (HFRS) is a group of clinically similar diseases that occur throughout Eurasia. HFRS includes diseases such as Korean hemorrhagic fever, epidemic hemorrhagic fever and nephropathia epidemica. HFRS begins as a flu-like illness and may progress to shock, bleeding, and renal failure. Mortality is 6 to 15%.

The research aimed to explore the changes of angiotensin-converting enzyme (ACE) blood activity and polymorphism A1166C (rs5186) of

the angiotensin II type 1 receptor as disease predictor at hemorrhagic fever with renal syndrome.

The studied groups included 409 patients with HFRS and 52 healthy controls. ACE blood activity was determined by Bühlmann ACE kinetic test. Genomic DNA was extracted from peripheral blood leukocytes by standard phenol-chloroform method. Genotyping was performed by the PCR-RFLP technique.

It was demonstrated that the ACE blood activity increased at HFRS and these changes were more significant at more severe form of disease. ACE blood activity depends on the form, and the period of HFRS: nonsignificant decrease is observed only in the feverish period of mild or severe form without complications. In severe form significant changes of ACE activity were observed, in complicated form - stable high activity during all disease. Analysis of angiotensin II type 1 receptor polymorphism A1166C demonstrated that neither *A1166 and *C1166 alleles nor *A1166/*A1166 and *C1166/*C1166 genotypes were associated with HFRS severity.

High ACE activity is not adaptive reaction due to defect in angiotensin II binding and it is an adequate metabolic response of an organism to endotheliotropic virus action.

J09.10 Genetic markers of predisposition to development of the metabolic syndrome in children with obesity.

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Increasing evidence suggests that there is genetic basis for the metabolic syndrome.

Objective: To study a contribution of candidate genetic markers in development of major metabolic syndrome components in children with obesity.

Study groups: 83 children aged 7-18 years old (13.3 ± 0.2 yr) with varying degree (I-IV) of obesity. Methods: Body mass index, waist circumference, blood pressure, and level of triglycerides, HDL-cholesterol, and glucose, were measured. ACE I/D, APOA1 G-75A, APOA5 S19W, APOC3 Sst1, APO E and W64R ADRB3 genetic polymorphism were identified using PCR-RFLP method.

Results: The genotype analysis showed that 33 out of 58 (57.0%) mono- and heterozygous carriers of D allele of ACE gene had a high blood pressure. More than half of heterozygous carriers of either alleles - 19W ApoA5 (11 children - 69.0%), -75A ApoA1 (15/56.0%), S2 ApoC3 (9/53.0%) and ε4 ApoE (20/83.3%) - presented with lipid disorders typical to metabolic syndrome (hypertriglyceridemia and/or hypoalphacholesterolemia). In 4 out of 10 (40.0%) children with RW genotype of ADRB3 gene presented with hyperglycemia and/or hypertriglyceridemia. It was found that the alleles accumulation (D ACE, 19W ApoA5,-75A ApoA1, S2 Apo C3 and ε4 ApoE) correlated with an increase in number of clinical criteria of metabolic syndrome ($r > 0.8$, $p < 0.01$).

Conclusion: Accumulation of rare alleles of genes determining lipid metabolism, level of blood pressure, and body mass index may be considered as molecular basis of genetic predisposition to extreme manifestation of metabolic syndrome.

J09.11 A New Research on Genetic Predisposition of Microtia: Endothelial Nitric Oxide Synthase (NOS3) and Myeloperoxidase (MPO) might be responsible of the ethiology of Microtia

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Objective. The aim of this study was to investigate the links between Endothelial nitric oxide synthase (NOS3) synthase, Myeloperoxidase (MPO) variants and microtia.

Methods. Nineteen microtia patients and 40 healthy controls were included in the study. We studied the polymorphisms of MPO (-463) and NOS3 (+894 polymorphism and VNTR in intron4) genes using the PCR and/or PCR-RFLP method.

Results. Significant differences were not determined for MPO (-463)

polymorphism from the genotype distribution and allele frequency between microtia and healthy control groups. In contrast, significant differences were determined in the NOS3 (+894 polymorphism and VNTR in intron 4) polymorphisms between microtia and healthy control groups ($P < 0.05$).

Conclusion. According to our literature searches our study is the first analyze of the polymorphisms of NOS3 (+894 polymorphism and VNTR in intron 4) on microtia patients. Our data suggest that NOS3 gene polymorphisms might play important role on the etiopathogenesis of Microtia in Turkish patients. The findings of the current study highlight the necessity for prospective longitudinal studies in elucidating the relative contributions of various factors in diseases with a multifactorial etiology where there is interplay among genetic susceptibility and exogenous factors.

J09.12 The polymorphism of cytokine genes in patients with multiple sclerosis

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The multiple sclerosis (MS) - the heaviest of known central nervous system diseases- is one of the most topical questions nowadays. The etiology of MS is complex, with strong evidence implicating both environmental and genetic causes. Many researchers admits that the leading importance in development of immunopathological process at MS is an infringement of balance proinflammatory (IL-1b, IL-2, IL-6, IL-12, TNFa) and anti-inflammatory (IL-4, IL-10, TNFb) cytokines. In this study we analyzed the associations of MS with polymorphisms of *IL6* (-572G>C), *IL10* (- 627C>A) and *IL12p40* (1159A>C) gene in Russian of Bashkortostan. DNA of 133 MS patients and 239 controls were analyzed by

polymerase chain reaction. It was revealed, that in MS patients the *IL6*(-572)*G/*G genotype frequency was higher than in controls (89.47% vs. 71.13%, $p < 0.001$, OR=3.45). Concerning polymorphism - 627C>A of *IL10* gene we couldn't find differences of the genotype and allele frequencies between MS patients and controls. The association analysis of *IL12p40* gene polymorphic marker with MS has shown that at male the *C/*C genotype is connected with the lowered risk of development MS (OR=0.12). Our study confirms the involvement of cytokines in MS development.

J09.13 SPI polymorphism of collagen I type gene as a marker of undifferentiated connective tissue dysplasia in children with myopia

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Axial myopia develops due to incorrect intrauterine development of the eye (congenital myopia) or the excessive growth of the eyeball. It occurs in course of different hereditary connective tissue syndromes but more often in undifferentiated connective tissue dysplasia. Collagen genes (COL) are candidate to associate with development of myopia. OBJECTIVE: to study the association of SPI polymorphism of gene COL1A1 and children myopia with dysplasia of connective tissue. STUDY POPULATION: 40 children (80 eyes) aged 5-18 years (15 girls, 25 boys). Clinical eye and dental examination and genetic testing of SPI polymorphism in COL1A1 were done. RESULTS: Myopia was diagnosed in 7 children (14 eyes): 5 children (10 eyes) with axial myopia 2,75-7,50 DPT and 2 children (4 eyes) with refractive myopia 3,25-6,50 DPT. Dental abnormalities were detected in 6 children and other bite abnormalities in 25 children. 9 children hadn't any abnormalities. Genotype distribution of COL1A1 revealed that SS genotype was in 1 child, Ss in 13 children and ss in 26 children. In these 26 children with genotype ss: 4 children had low and middle degree of myopia. Among children caring Ss genotype 2 children had middle and severe myopia in the absence of dental abnormalities, 2 children with the same genotype had bite abnormalities, 1 child had a severe myopia 7,0 DPT and dental abnormalities. CONCLUSIONS: We hypothesize that the polymorphism in genes of collagen can influence on appearance of myopia with dental abnormalities and may be with other markers of

undifferentiated connective tissue dysplasia.

J09.14 Psychological aspects of longevity and their genetic correlates

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Psychological functioning of 150 healthy patients of gerontological clinic, aged 60 to 94 years, was studied ($m=76$), on the levels of neuroticization (both reactive and personal), psychological defence mechanisms (negation, exclusion, regression, compensation, projection, replacement, intellectualization, reactive formation), and intrinsic religiosity (along with features of altered states of consciousness). Simultaneously a genetic survey was carried out, directed at detection of I/D polymorphism of angiotensin-converting enzyme (ACE), and A1/A2 (C102T) polymorphism of gene of serotonin receptor 5HT2A. Correlation between ACE and neuroticization was demonstrated by means of factor analysis, as well as with functioning of the cardiovascular system. Another correlation linked serotonergic system with the age of the patients, as well as the level of their intrinsic religiosity. In this way, existence of links between the genetic basis of longevity on the level of major functional systems, and psychological processes, peculiar for it, was corroborated. The study was supported by Russian Foundation for basic Research, grant 09-06-00012a; and by Program of Basic Research of the Presidium of Russian Academy of Sciences 'Fundamental Sciences for Medicine'.

J09.15 The study of CYP21A2 gene mutations in Russian woman with recurrent miscarriages

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Recurrent miscarriage (RM) which is known as one of the three or more repeated and spontaneous pregnancy losses represents an actual problem of modern obstetrics. The mild, nonclassic form of the congenital adrenal hyperplasia (CAH) is known as a common cause of RM (about 25%). CAH is a common autosomal recessive disease most frequently due to mutations in the CYP21A2 gene. CAH is responsible for different forms of virilization of external genitalia in newborn girls and, in its most severe form, if not treated properly can be lethal. Patients with the mild, nonclassic form of the disease have less severe symptoms complicated with the symptoms of postnatal androgen excess. Moreover, mutations of CYP21 can lead to various reproductive disorders in females so they can be found in RM patients. By means of PCR and RFLP analysis 36 unrelated females with RM and 48 unrelated donors were screened for 11 the most common mutations of CYP21A2 gene including gene deletion (*delA2*), large gene conversions (including 2 or 3 exons), *G110del8nt*, *P30L*, *I2splice*, *I172N*, *V281L*, *Q318X*, *R356W*, *E6cluster*, *P453S*.

Mutant alleles were detected in 1 (2%) donor (gene conversion, including *V281L* and *Q318X*) and in 6 (17%) RM patients. 3 *Q318X* heterozygotes and 3 heterozygotes for three other mutations (*I2splice*, *R356W*, *delA2*) were registered. Thus according to our data CYP21A2 mutations are responsible for the significant proportion of recurrent miscarriages. Analysis of CYP21A2 mutations in the females with RM could be recommended for revealing patients with the mild, nonclassic form of CAH.

J09.16 Are Endothelial Nitric Oxide (NOS3) gene polymorphisms important in Rheumatoid Arthritis ?

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Objective: Rheumatoid arthritis (RA) is a chronic inflammatory joint destruction. Endothelial nitric oxide synthase (NOS3) is involved in key steps of immune response. The aim of this study was to explore the association between NOS3 gene polymorphisms and clinical param-

eters in patients with RA.

Methods: Genomic DNA was obtained from the peripheral blood of 65 patients with RA and 70 unrelated healthy controls. We genotyped NOS3 polymorphisms (+894, intron 4 VNTR) using PCR and/or PCR-RFLP method. Also clinical parameters, demographic data of the patients and response to drug treatment were recorded. All data were analyzed using SPSS version 14.0 for windows.

Results: NOS3 (+894) TT genotype frequencies were significantly higher among RA patients ($p=0.029$ for TT) than control ones, whereas no associations with NOS3 (VNTR) polymorphisms. The observed genotype counts was not deviated from those expected according to the Hardy-Weinberg Equilibrium ($p>0.05$). A significant association was detected between these polymorphisms and response to treatment in anti TNF α treatment subgroup (improvement in DAS28 scores in RA patients anti-TNF α and DMARD treatments) ($p=0.004$).

Conclusions: In the present study, we made an investigation to establish to the best of our knowledge for the first time an association between two well-established polymorphisms of the NOS3 gene polymorphisms and clinical parameters in RA. The present study showed that two common polymorphisms of the NOS3 gene were significantly associated with occurrence of RA.

J09.17 The genetics of Suicide Behaviour

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Suicide is one of the leading causes of death worldwide (Giegling et al., 2009). Guided by the twin-, adoption-, and family-based evidence suggesting the existence of genetic diathesis for suicidal behaviors, molecular studies began to look for specific genetic components of this diathesis. The candidate-based association approach has been the dominant strategy, and close to 50 candidate genes have been investigated. Spurred by the wealth of neurobiologic knowledge, molecular geneticists focused primarily on neurotransmitter systems and less on genes outside of these systems.

Over the past 30 years, indirect evidence for the existence of a genetic component in the suicidal diathesis has come largely from family, twin, and adoption studies.

In order to understand if there was association between suicide behaviour and genetic factors, we choose 6 isolated populations located in North Italy, in Friuli Venezia Giulia.

Suicidal ideation was defined on the Hamilton Rating Scale for Depression (HRSD; Hamilton, 1960), it is a widely used interviewer-administered measure of the depressive symptom severity. The HRSD suicide item consists of 4 ratings of suicidal behavior: 0 ("absent"), 1 ("feels life is not worth living or any thoughts of possible death to self"), 2 ("wishes he were dead"), 3 ("suicidal ideas or gestures"), or 4 ("attempts at suicide").

We analysed and genotyped 871 people and the age range was from 18 years old to 65 years old (Akyuz et al. 2005). Whole-genome genotyping (Illumina - Infinium) and association analysis (ProbABEL) showed promising preliminary results.

P10 Evolutionary and population genetics, and Genetic epidemiology

P10.01 From the diagnosis of Autosomal Recessive disorders to the identification of "clusters" of affected patients, up to population prevention programs

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In Italy, as well as in other Countries of the old Europe, the Diagnosis of Genetic Disorders with Autosomal Recessive inheritance often concerns villagers of small communities, quite closed and with high rate of consanguinity, due to historical and geographical reasons. The identification of real clusters of subjects affected by specific AR disorders must be carefully pursued to realize prevention programs at population levels, by the identification of heterozygote individuals.

Here we report three examples of clusters identified in different Italian regions, starting from a first patient referred for a clinical genetic evaluation.

In the geographical area of Brindisi (Puglia region) we made diagnosis of 3M dwarfism in a child of consanguineous parents. Ten other affected people were later identified, by clinical and molecular screening of CUL7 gene. The number of identified heterozygotes is increasing thanks to a collaborative program with the local Medical Doctors.

In the Basilicata region, we made diagnosis of Roberts syndrome (cohesinopathy), in two subjects of the same sibship, with consanguineous parents. The molecular screening of ESCO2 gene allowed identification of a "local" mutation in a small geographical area.

The third cluster of patients affected by a metabolic disorder, the Mucolipidosis III, was identified in Calabria, in a little village with less than 100 inhabitants, and about 90% of families with the same surname. Our clinical and molecular study was stimulated by the diagnosis of this disorder in two "unrelated" girls, with different clinical expression but considerably increased levels of lysosomal hydrolases.

P10.02 ACE I/D polymorphism and human longevity - Croatian senescence study

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The insertion/deletion (I/D) polymorphism of the angiotensin-1-converting enzyme gene (ACE) has been suggested to be associated with disease and longevity but the findings are confounding. There are indications that the ACE DD genotype predisposes to several cardiovascular diseases including hypertension but it is more frequent in the aging populations. We studied ACE I/D polymorphism and hypertension in Croatian senescent population (85-101 yrs; N=300) where we found no genotype association with hypertension, while earlier results in young Croatian adults (18-40 yrs) showed such relationship for DD genotype. Of the investigated risk factors for hypertension, age, female sex (OR=2.88; 95%CI=1.49-5.56) and triglyceride concentration showed significant influence, while BMI and cholesterol concentration did not. Further, we tested the association between ACE I/D polymorphism and longevity in Croatian population. For comparison we used previously reported frequencies for the general Croatian population aged 18-80 yrs. The genotype and allele distributions differed significantly between two age cohorts with DD genotype being more frequent in senescents than in the younger cohort (42.5% vs. 22.1%), and with D allele frequencies of 61.0% vs. 49.4%. This finding supports the association of ACE I/D polymorphism with longevity while controversial results have been obtained in other European populations. The established ACE I/D allele and genotype frequencies fit into the pattern of increasing gradient of D and DD frequencies in senescents from Northern to Southern Europe. Such genetic differences indicate that D allele - in addition to being CVD risk factor - might have some unrecognized advantageous role in successful human ageing.

P10.03** Clear detection of ADIPOQ locus as the major gene for plasma adiponectin: results of genome-wide association analyses including 4659 European individuals

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Objective. Plasma adiponectin is strongly associated with various components of metabolic syndrome, type 2 diabetes and cardiovascular outcomes. Concentrations are highly heritable and differ between men and women. We therefore aimed to investigate the genetics of plasma adiponectin in men and women.

Methods. We combined genome-wide association scans of three population-based studies including 4659 persons. For the replication stage in 13795 subjects, we selected the 20 top signals of the combined analysis, as well as the 10 top signals with p-values less than 1×10^{-4} for each the men- and the women-specific analyses. We further

selected 73 SNPs that were consistently associated with metabolic syndrome parameters in previous genome-wide association studies to check for their association with plasma adiponectin.

Results. The *ADIPOQ* locus showed genome-wide significant p-values in the combined ($p=4.27 \times 10^{-24}$) as well as in both women- and men-specific analyses ($p=8.66 \times 10^{-17}$ and $p=2.49 \times 10^{-11}$, respectively). None of the other 39 top signal SNPs showed evidence for association in the replication analysis. None of 73 SNPs from metabolic syndrome loci exhibited association with plasma adiponectin ($p>0.01$).

Conclusions. We demonstrated the *ADIPOQ* gene as the only major gene for plasma adiponectin, which explains 8.7% of the phenotypic variance. We further found that neither this gene nor any of the metabolic syndrome loci explained the sex differences observed for plasma adiponectin. Larger studies are needed to identify more moderate genetic determinants of plasma adiponectin.

P10.04 Concordance of different genetic markers in human population studies

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A polymorphism of 10 Alu loci (A25, ACE, APO, B65, D1, FXIIIB, HS2.43, HS4.65, PV92 and TPA25), 15 autosomal STR loci (D3S1358, TH01, D21S11, D18S51, PENTA E, D5S818, D13S317, D7S820, D16S539, CSF1P0, PENT.D, vWA, D8S1179, TPOX, FGA) and 6 blood groups (Rh, MN, Duffy, Kidd, KELL and Lutheran, genotyped using PCR-RFLP and PCR-SSP methods) have been observed. Also, polymorphism of 10 phenotypic traits (red colorblindness, green colorblindness, PTC testing, digital index, ear lobe type, midphalangeal hair, crooked little finger, tongue rolling, thumb distal extensibility, thumb proximal extensibility) have been estimated within 300 individuals in human populations placed in 10 regions across Bosnia and Herzegovina.

Main goal was to compare validity of human population genetics analyses implementing different type of genetic markers.

Classical population genetic analyses have been performed, such as deviation from Hardy-Weinberg equilibrium, genetic diversity, Fst and genetic distance. Neighbor-Joining trees were constructed based on results of genetic distance analyses. In order to compare results of analysis based on different molecular and phenotypic markers we have implemented Mantel test for comparison of genetic distance results, then the statistical analysis for genetic diversity and genetic differentiation comparisons, as well as inter-rater reliability test. Trees constructed on the basis of the different observed markers traits were compared using the tree distance methodology. Modest correlation between results of population genetics analyses based on different markers has been detected specially between biallelic markers. Strong and clear correlation between biallelic and multiallelic markers has not been detected.

P10.05 Allele frequencies of apolipoprotein E gene polymorphisms in western romanian population

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The aim of this study was to establish the frequency of apoE alleles ($\epsilon 2$, $\epsilon 3$ and $\epsilon 4$) and the genotypes in elderly patients (age > 60 years) with symptoms suggestive for Alzheimer's disease, from western Romania.

Method. 30 patients were selected based on patients' history, collateral history from relatives, and the presence of characteristic neurological and neuropsychological features. For the genotyping we used Real-Time PCR and melting curve analysis (ApoE ToolSet for LightCycler, Roche).

Results. We identified 58 apoE $\epsilon 3$ alleles (96,66%) and 2 apoE $\epsilon 4$ alleles (3,33%). For two patients we had a heterozygous genotype ($\epsilon 3/\epsilon 4$, 6,66%) and for the rest of the patients the genotypes were homozygous ($\epsilon 3/\epsilon 3$, 93,33%). There was a good correlation between the heterozygous genotypes of the two patients and their symptomatology, however, even if the diagnostic of Alzheimer's disease can not be excluded for the rest of the patients based only on apoE genotyping, for genetic investigation we need a more thorough selection of patients. Also the absence of the risk apoE allele, $\epsilon 4$, in correlation with other clinical and imagistic findings, may impose for some patients a change of diagnosis.

P10.06** Genome-based prediction of breast cancer risk in the general population: a modeling study based on meta-analyses of genetic association studies

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Background Genome-wide association studies have identified novel susceptibility variants for breast cancer, which potentially can be used to predict breast cancer in asymptomatic women. This review and modeling study aimed to investigate the current and potential predictive performance of testing at multiple genetic loci (genetic profiling).

Methods Genetic profiles were simulated for a population of 10,000 women, in which we assumed a breast cancer lifetime risk of 10%. Genetic profiles were based on polymorphisms from meta-analysis including, in separate scenarios, all polymorphisms or statistically significant polymorphisms only. We additionally investigated the magnitude of the odds ratios (OR) for 1 to 100 hypothetical polymorphisms that would be needed to achieve similar discriminative accuracy as available prediction models (modeled range of area under the receiver operating characteristic curve [AUC] 0.65-0.75).

Results Of the 71 polymorphisms that had been investigated in meta-analyses, 26 showed nominally significant associations. AUC was 0.64 for genetic profiles based on all 71 polymorphisms and 0.62 for the 26 significant polymorphisms. Addition of 50 additional variants, each with risk allele frequencies of 0.30, would require an OR of 1.2 to increase this AUC to 0.65, 1.3 to increase AUC to 0.70 and 1.4 to increase AUC to 0.75. To achieve AUC of 0.75, even 100 additional variants would need ORs of 1.3 to 1.5 per allele, depending on risk allele frequencies.

Conclusion A substantially large number of genetic susceptibility variants need to be discovered and validated to improve the predictive performance beyond that of current breast cancer risk models.

P10.07

Estimation of CFTR mutation carrier frequency based on known frequency of p.F508del in Iranian neonates

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Cystic fibrosis is the most common autosomal recessive disease in many Caucasian populations. Approximately one in 2500 newborns in populations of European ancestry are affected, wherein the average carrier frequency is 1:25. The disease is caused by mutations in *CFTR* gene. Among the more than 1600 mutations identified in the causative *CFTR* gene, the p.F508del allele is the most common worldwide. Among mutated alleles, the frequency of this allele exhibits a northwest to southeast gradient, ranging from a high of 88% in Denmark to a low of approximately 16% in Iran. In the present study, we screened for p.F508del mutation in the DNA of 800 placental cord blood samples obtained from newborns from 4 geographical regions in Iran. Based on the number of carriers identified and the observation that this allele accounts for 16% of the *CFTR* mutated alleles of the Iranian population, the frequency of carriers of *CFTR* mutations in Iran was calculated to be 0.037 (95% confidence level of 0.037 +/- 0.016). This figure corresponds to 1:27, very close to the carrier frequency of European populations. This finding suggests that CF, contrary to conventional thinking, is a disease of considerable public health importance in Iran, and probably in other countries of the Middle East.

P10.08 Study on prevalence of cleft lip and cleft palate and effect of consanguinity among Iranian population

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Background: Clefts of the lip and palate are one of the most common congenital birth anomalies. Genetic factors play a great role in the etiology of them and the high percentage of the consanguinity of the parents of the affected persons is one of the reasons. These defects not only make abnormal changes on appearance of the neonate, but make a lot of stress and psychological problems for the patients and

their families. Study on the prevalence of clefts, their risk factors and also genetic counseling for affected persons and their families can be a guideline for general population and probably reduce these anomalies over the generations.

Methods: A total of 7374 pedigrees of all the patients admitted to the Department of Genetic, were studied during 2002-2005 and 99 pedigrees with the patients with cleft lip ± palate or isolated cleft palate were separated. The total number of cases among these 99 pedigrees was 136. The effects of consanguinity, positive family history and sex were investigated among cases.

Results: 70.8% of patients with syndromic clefts and 58.7% of patients with nonsyndromic CL±P had parents with consanguineous marriage. In addition 44.4% of patients with nonsyndromic CL±P had positive family history.

Conclusion: In our population prevalence of nonsyndromic CL±P (95%CI) is estimated to be 7 in 1000 (5,9) and prevalence of non-syndromic CP (95%CI) is estimated to be 3.1 in 1000 (1.8,4.4) and consanguinity of parents seems to have a significant role ($p=0.02$) on prevalence of clefts.

P10.09 Recurrence of congenital heart defects in families

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Background Knowledge regarding the familial contribution to congenital heart defects (CHD) on an individual and population level is sparse. We estimated an individual's risk of CHD given a family history of CHD, and the contribution of CHD family history to the total number of CHD in the population.

Methods and Results In a national cohort study, we linked all Danish residents to the National Patient Register, the Causes of Death Register, the Danish Central Cytogenetic Register, and the Danish Family Relations Database, yielding 1,763,591 persons born in Denmark, 1977-2005 of whom, 18,708 persons had CHD. Individuals with CHDs were classified by phenotype. We estimated recurrence risk ratios (RRR) and population attributable risk. Among first degree relatives, RRR for heterotaxia was 79.1 (95% confidence interval 32.9-190), conotruncal defects 11.7 (8.0-17.0), atrioventricular septal defect 24.3 (12.2-48.7); left 12.9 (7.48-22.2) and right ventricular outflow tract obstruction 48.6 (27.5-85.6), isolated atrial septal defect 7.1 (4.5-11.1), and isolated ventricular septal defect 3.4 (2.2-5.3). The overall RRR for same defect was 8.15 (6.95-9.55), whereas it was 2.68 (2.43-2.97) for different heart defects. Only 2.2% (4.2% when excluding chromosomal aberrations) of CHD in the population were attributed to CHD family history in first degree relatives.

Conclusions Specific CHDs showed highly variable but strong familial clustering in first degree relatives, ranging from 3-fold to 80-fold compared to the population prevalence, whereas the cross-over risks between dissimilar CHD were weaker. Family history of any CHD among first degree relatives accounted for a small proportion of CHD in the population.

P10.10 Inferences from the proportion of compound heterozygotes among affected offspring of consanguineous parents

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It is well known that consanguineous parents have an increased risk of autosomal recessive diseases in their children. Although most of the affected children will show mutations in a homozygous state, some do present with two different mutations of the gene concerned.

We wondered what, if any, inferences could be made from the proportion of these compound heterozygotes among affected children of consanguineous couples.

We showed that knowledge of (a) this proportion, (b) the inbreeding coefficient, and (c) the number and relative frequency of pathogenic alleles will be sufficient to calculate: (1) the proportion of homozygotes identical by descent, and (2) the proportion of homozygotes non-identical by descent among affected offspring of consanguineous parents,

as well as (3) the sum frequency of pathogenic alleles of a particular gene in the population. The last parameter can be used to calculate the prevalence of the disease and of carriers in the population.

The equation to calculate the sum frequency of pathogenic alleles (q) in the population is

$$q = [P(CH) \cdot (F + q \cdot Fq)] / [(1-F)(1 - \sum a_i^2)],$$

in which P(CH) is the proportion of compound heterozygotes, F is the inbreeding coefficient, and a_i is the relative frequency of the i^{th} allele. When the necessary data (a-c) are available, e.g. in a clinical genetic laboratory, the method avoids the workload and pitfalls of epidemiologic surveys. Limitations are broad confidence limits and complications resulting from population structure.

P10.11 Association study of 16 susceptibility loci with Crohn's disease in Russian population

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Crohn's disease (CD) is a chronic relapsing inflammatory bowel disorder which is thought to result from a complex interplay of multiple genes and environmental factors. At present the incidence of this disorder increases in the world. Therefore CD remains to be one of the serious problems in the gastroenterology. Genome-wide association studies have recently identified more than 30 susceptibility loci for Crohn's disease. We performed a replication study of 16 SNPs of 15 genes (PTPN22, IL-23R, IL-10, IL-18RAP, ATG16L1, MST1, IBD5 locus, IL-12B, IRGM, BTNL2, JAK2, TNFSF15, NKX2-3, STAT3, PTPN2) in 113 Russian CD patients and 138 controls using TaqMan Assays (Applied Biosystems). A selection of SNPs was made based on the data from the original genome-wide association studies. The patients and the controls were recruited in the North-West region of Russian Federation. We identified the association of the JAK2 rs10758669 (A>C) polymorphism with Crohn's disease in Russian population. The frequency of C-allele was significantly higher in CD patients ($p=0.018$, OR=1.60, 95% CI=1.10-2.33). The carriers of homozygous C/C or heterozygous A/C genotypes had the 2-fold increased risk of having CD as compared with the carriers of homozygous A/A genotype (OR=2.07, 95% CI=1.22-3.52, $p=0.0082$). Thus, C-allele of JAK2 rs10758669 (A>C) polymorphism could be the risk factor of CD in Russian population.

P10.12 Pharmacogenetics of coumarins: prevalence of CYP2C9 and VKORC1 polymorphisms in the Lebanese population

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Polymorphisms in the genes encoding the cytochrome P450 2C9 (CYP2C9) and the Vitamin K epoxide reductase (VKORC1) contribute to the variability in sensitivity to coumarins. Patients with common genetic variants of CYP2C9 (*2 & *3) or VKORC1 (-1639A allele) require a lower dose of coumarin, a longer time to reach a stable dose and are at higher risk of serious bleeding.

No prevalence for those variants has so far been reported in the Lebanese population. CYP2C9 (*1/*2/*3) and VKORC1 (A/G) were assessed by Polymerase Chain Reaction-Restriction Length Polymorphism in 150 unrelated healthy Lebanese volunteers, representing the various geographic regions of Lebanon.

We found no significant difference in the frequency of CYP2C9 variants and other evaluated Caucasians. However, the frequency of homozygotes for the VKORC1 -1639A was appreciably higher, a finding of interest, to be correlated with clinical risk.

| | Lebanese | Caucasian | Black | Asian |
|--------|------------------|-----------|-------|-------|
| CYP2C9 | *1 / *1 100 % | 70 % | 96 % | 99 % |
| | *1 / *2 15 % | 29 % | 3 % | 0 % |
| | *1 / *3 15 % | | | |
| | *2 / *2 1 % | | | |
| | *2 / *3 1 % | 4 % | 0 % | 0 % |
| | *3 / *3 1 % | | | |
| VKORC1 | G / G 25 % | 43 % | 72 % | - |
| | G / A 42 % | 45 % | 28 % | - |
| | A / A 34 % | 12 % | 0 % | - |
| | | | | |

P10.13 Spectrum of *CFTR* mutations in CF-patients in Siberia region of Russia

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Cystic fibrosis (CF) is a heterogeneous and the most common autosomal recessive disorder. We report the results of *CFTR* molecular screening of CF-patients referred to the Institute of Medical Genetics (Tomsk - Russia) in the last five years. The DNA samples of 128 unrelated patients affected by cystic fibrosis, classical form, living in Siberia region of Russia had been analyzed. Patients had been tested for mutations: F508del, I507del, 1677delTA, CFTRdele2,3 (del21kb), R334W, R347P, G551D, R553X, G542X, 2143delT, 2184insA, 394delTT, 306delTAGA, 3821delT, L138ins, N1303K, W1282X in the *CFTR* gene. In group of 128 tested patients (256 alleles) 86 patients (67,2%) were either homozygous or compound heterozygous, 41 patients (32,0%) carried only one detected mutation. Allelic frequencies for tested mutations were: 60,55% (F508del), 7,81% (CFTRdele2,3 (del21kb)), 5,47% (2184insA), 1,95% (R334W), 1,56% (N1303K), 1,17% (2143delT) and 1,17% (394delTT), 0,78% (R347P) and 0,78% (W1282X), 0,39% (G542X), 0,39% (R553X), 0,39% (3821delT). We found one novel for Siberia region mutation - 2-bp deletion in exon 20 (*CFTR* gene) - 3944delGT. We supposed the presence of novel mutated allele in two patients due to unusual profile of restriction fragments (during the W1282X-mutation analysis); and subsequently *CFTR* mutation - 3944delGT - was determined by sequence analysis of PCR-products in DNA of these two CF-patients from different regions of Siberia. And since we had included testing of 3944delGT mutation in our *CFTR*-mutation panel for screening DNA from patient affected by cystic fibrosis and for prenatal diagnosis in CF-risk families in Siberia region of Russia.

P10.14 Study of DFNB59 gene (Pejvakin) promoter mutations associated with deafness in deaf subjects of Chaharmahal va Bakhtiari province in Iran

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Background and aims: Hearing loss is a very heterogeneous disorder and occurs due to genetic, environmental or both causes. More than 100 genes have been predicted to cause deafness. More recently a gene called DFNB59 has been shown to cause neural deafness in 4 Iranian families. This study aims to investigate the frequency of DFNB59 promoter mutations in a cohort of 100 deaf subjects in Chaharmahal va Bakhtiari province.

Materials and Methods: One hundred nonsyndromic hearing loss subjects were investigated for presence of DFNB59 promoter mutations. DNA was extracted by phenol chloroform protocol. The mutation screening of the gene was performed using PCR-SSCP procedure and subsequent direct sequencing.

Results: No mutation was detected in promoter of the DFNB59 in deaf individuals studied.

Conclusion: We conclude that the promoter mutations of the gene have no contribution in causing deafness in samples studied. However to find the cause of deafness in this province, other genes or loci need to be investigated in the future.

P10.15 Interaction between long-term air pollution exposure and genes in relation to levels of inflammatory markers

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Background: Air pollution exposure can lead to adverse cardiovascular effects, possibly via systemic inflammation and altered coagulation balance. Genetic variation may play a role in susceptibility to these effects.

Objectives: To investigate the modifying capacity of genetic variants in genes related to inflammation (*IL6*, *TNF*) and coagulation (*fibrinogen Bβ*, *PAI-1*) on the effects of long-term exposure to air pollution on blood marker levels.

Methods: We studied a population sample of 1013 men and 493 women aged 45-70 years from Stockholm. Spatial modelling was used

to assess long-term air pollution exposure to traffic-related NO_2 and heating-related SO_2 emissions at the home address of each individual over retrospective periods of up to 30 years. We investigated polymorphisms in *IL6* (-598GA, -573GC, -174GC), *TNF* (-1031TC, -863CA, -857CT, -308GA, -238GA), *fibrinogen Bβ* (-455GA) and *PAI-1* (-675 4G/5G).

Results: Gene-environment interactions were observed for several *IL6* and *TNF* SNPs. For example, one-year traffic- NO_2 exposure was associated with levels of IL-6 in homozygote *IL6*-598AA carriers (+266%; 95%CI +5% to +744%) per 28.1 $\mu\text{g}/\text{m}^3$ (difference between the 5th and 95th percentile of exposure), but not in -598GG/GA carriers (+16%; 95%CI -26% to +80%). One-year heating- SO_2 exposure was associated with levels of TNF- α in homozygote *TNF*-308AA carriers (+164%; 95%CI +15% to +503%), but not in -308GG/GA carriers (-6.5%; 95%CI -20% to +9%) per 6.8 $\mu\text{g}/\text{m}^3$.

Conclusions: Genetic variants in *IL6* and *TNF* genes may modify the effect of long-term exposure to moderate levels of air pollution on the levels of inflammatory blood markers in healthy subjects.

P10.16 Single Nucleotide Polymorphisms in the NOS2 and NOS3 Genes are Associated with Exhaled Nitric Oxide

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Background: Fraction of Exhaled Nitric oxide (FENO) is a non-invasive biomarker of airway inflammation. Nitric oxide (NO) is an endogenous molecule synthesized from three distinct NO synthase genes (NOS1, NOS2 and NOS3), and plays an important role in pathophysiology of airway diseases. The genetic regulation of NO and its influence on FENO is not yet well understood.

Aim: To comprehensively investigate Single Nucleotide Polymorphisms (SNPs) in the NOS genes and association with FENO.

Method: A population-based study of 2200 randomly selected adults was conducted 2001–2003 in Gothenburg, Sweden. FENO was measured at a flow-rate of 50 mL/s. We genotyped 20 NOS1, 17 NOS2 and 12 NOS3 tag SNPs. We used linear regression models to estimate effects of NOS genotypes on FENO. Models were adjusted for age, sex, height, atopy and smoking habits.

Results: The mean FENO level for all subjects was 14.9 parts per billion. In a multi-SNP model, two SNPs in NOS2 (rs9901734 [C/G] and rs3729508 [T/C]) and one SNP in NOS3 (rs7830 [G/T]) were significantly associated with higher levels of FENO₅₀. For rs9901734, subjects had 5.3% higher levels of FENO₅₀ per G allele ($p = 0.016$); for rs3729508, subjects with CC or CT genotypes had 9% higher levels compared with TT ($p = 0.0057$); and for rs7830, subjects with GT or TT had 5.3% higher levels than GG ($p = 0.0485$).

Conclusion: Based on thorough genotyping of the three NOS genes, polymorphisms in the NOS2 and NOS3 genes were independently associated with inflammation in the airways, as measured by FENO₅₀.

P10.17 Polymorphisms of folate related genes and a risk of having a child with chromosome aneuploidy

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Aneuploidy is the most common chromosome abnormality in humans and is the leading genetic cause of miscarriage and congenital birth defects. Several studies indicated that polymorphisms in folate-related genes can lead to DNA hypomethylation and abnormal chromosomal segregation. The aim of this study was to investigate the possible association of four polymorphisms in the three folate-related genes (MTHFR C677T, MTHFR A1298C, MTR A2756G and MTRR A66G) with a risk of having a fetus with chromosome aneuploidy. We studied a total of 48 parents with a fetus with trisomy 21 (n=250, trisomy 18 (n=9), trisomy 13 (n=3), 45, X0 (n=5), 47, XXY (n=3) and triploidy (n=3). The parental origin of the aneuploidy was determined by QF-PCR analysis of several STR markers on each affected chromosome in the fetus and both parents. The MTHFR C677T, MTHFR A1298C, MTR A2756G and MTRR A66G were genotyped by multiplex PCR followed by SNaPshot analysis. The distribution of the allele and genotype frequencies of the four studied folate polymorphisms in the parents from whom the aneu-

ploidy originated were compared with the frequencies in their partners as well as in ethnic matched individuals from the general population. No difference was observed in the allele and genotype frequencies of the four studied folate polymorphisms between the parents from whom the aneuploidy originated and the control groups. In conclusion, our study do not support the relationship between the four studied folate polymorphisms and a risk of having a child with an aneuploidy.

P10.18 Distribution of FMR1(CGG)_n repeats and FRAXAC1/DXS548 alleles in healthy population of Croatia

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Fragile X syndrome is caused by expansion of a (CGG)_n trinucleotide repeat within the 5' untranslated region of the FMR1 (fragile X mental retardation 1) gene transcript. The aim of our study was to report the distribution of FMR1 (CGG)_n alleles and DXS548-FRAXAC1 haplotypes in a healthy Croatian population. EDTA blood samples were collected from healthy, unrelated individuals from Croatia. We analyzed a total of 452 (680 X chromosomes) healthy individuals (224 males and 228 females) using PCR and electrophoresis on a 6% polyacrylamide gel in an automated sequencer (ALFexpress). Investigation of the FMR1 alleles in a sample of the Croatian population revealed that 98.4% alleles were in the normal range (less than 40 CGG repeats). We also identified seven intermediate-sized alleles (40-54 CGG repeats). No carriers with premutations (55-200 CGG repeats) were observed in this sample. The most common CGG repeat allele was 29 allele (20.0%), followed by 28 allele (14.4%). We evaluated the FRAXAC1 and DXS548 polymorphism in 366 X chromosomes of 200 healthy subjects (60 males and 153 females). Five different FRAXAC1 alleles were observed, with a predominance of the FRAXAC1-3 (154pb) allele (66.9%). For DXS548, there were seven different alleles observed with a predominance of the DXS548-7 (194pb) allele (81.7%). The present study provides information about the distribution of FMR1 alleles in our population and represents foundation for all future studies of the fragile X syndrome among Croatians.

P10.19 G-308A polymorphism in TNF gene in different regions of Buryatia

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G-308A polymorphism in the tumor necrosis factor-alpha (TNF) gene has been studied in the Buryat population by genotyping samples from 16 subpopulations from 5 areas of Republic Buryatiya (total N=865). The TNF-alpha G-308A polymorphism was assessed by the PCR and restriction analysis. It has been shown that mean frequency of allele A in Buryats was 11.5 %. At comparison with other Siberian populations, statistically significant differences with Yakuts were found. The frequency of this polymorphism in Siberian populations (8-19%) is higher than in populations of East Asia (2-6%). In three Buryat subpopulations of 16 studied, the deviation of genotype frequencies from Hardy-Weinberg equilibrium has been found. Genetic differentiation coefficient which was calculated for all settlements ($Gst=0.0167$) and for 5 investigated areas ($Gst=0.0053$), did not demonstrate significant differentiation of Buryat population by the given polymorphism. The results describe the prevalence of functionally significant polymorphism in TNF gene, which is associated with various common diseases and endophenotypes.

P10.20 GCKR gene functional variants in patients with type 2 diabetes and metabolic syndrome: do the rare variants also associate with an increased carotid intima-media thickness in metabolic syndrome?

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Background and Aims: Here we analyzed primarily the association of two glucokinase regulatory protein (GCKR) variants (rs780094 and rs1260326) with triglyceride and glucose levels in Hungarian type 2 diabetes mellitus and metabolic syndrome patients; and also correlated the genotypes with the pooled carotid intima-media thickness records.

Methods and Results: A total of 321 type 2 diabetic patients, 455 metabolic syndrome patients, and 172 healthy controls were genotyped by PCR-RFLP. Both GCKR variants were found to associate with serum triglycerides and with fasting plasma glucose. However, significant association with the development of type 2 diabetes mellitus and metabolic syndrome could not be observed. Analyzing the records of the patients, a positive association of prevalence the GCKR homozygous functional variants and carotid intima-media thickness was found in the metabolic syndrome patients.

Conclusion: Our results support that rs780094 and rs1260326 functional variants of the GCKR gene inversely modulate serum triglycerides and fasting plasma glucose levels, as it was already reported for diabetic and metabolic syndrome patients in some other populations. Besides this positive replication, as a novel feature, our preliminary findings also suggest a cardiovascular risk role of the GCKR minor allele carriage based on the carotid intima-media thickness association.

P10.21 Medical genetically study of hereditary disorders in European part of Russia

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Based on a medical genetically study, the genetic diversity of AD, AR and X-linked recessive hereditary disorders (HDs) in 12 regions and ethnic groups of Russia were established: Adygean, Maris, Chuvashes, Udmurt, Bashkirs and Russian from seven populations. The size of the investigated populations was than 3 million inhabitants. All population was examined by standard protocol of medical genetic research elaborated in laboratory of genetic epidemiology, Research Centre for Medical Genetics. About 2500 HDs of OMIM could be identified by this research. Clinical investigations were performed by a geneticist, a neurologist, an ophthalmologist and other physicians, focused on diagnostic of HDs. In the 12 Russian regions surveyed, we revealed a total of 460 (217 AD, 185 AR and 58 X-linked) clinically differing HDs (more than 7000 affected). In order to determine the geographic location of various populations and ethnic groups in a variety of the studied populations of Russia by the prevalence rate (10-3) of AD and AR diseases, we performed the cluster analysis with the use of the average linkage method. Analysing the dendograms showed, that first, all Russian populations are united into a single cluster, followed by the addition of the cluster of the Finno-Ugric population and the Chuvashian population. The greatest genetic remoteness by genes of HDs was detected for the Bashkirs and the Adygeans. It should be mentioned that the obtained pattern of genetic interrelationships between the studied populations/ethnic groups is largely similar to that obtained while using conditionally neutral polymorphic genetic systems.

P10.22 Y-chromosomal background of gr/gr deletions

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The gr/gr deletion is a common Y chromosome abnormality that removes 1.6Mb of the AZFc region including two DAZ and one CDY1 gene copies. The relationship between this deletion and male infertility is a subject of a continuing intense debate. The gr/gr deletion is regarded as a risk factor for reduced sperm counts in some populations, but not in others. We screened a total of 740 men from the Republic of Macedonia (520 Macedonians, 140 Albanians and 80 Roma, Serbs, Turks and Croats) for the presence of gr/gr deletion. 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 21 men with gr/gr deletion and gr/gr deletion-b2/b4 duplication; 19 Macedonians, one Serb and one Croat. No gr/gr deletion was detected among Albanians. Nine different Y chromosome haplogroups were determined

among men with gr/gr deletions, three of which (I2a-P37b, R1a1-SRY1532 and J2b2-M241) are among the five most common haplogroups in the Republic of Macedonia. The most frequently deleted DAZ copy was DAZ1/2, whereas the predominantly missing CDY1 copy was CDY1b. There was a difference in the haplogroup distribution of carriers with the loss of CDY1a and CDY1b. The ethnic difference in the incidence of gr/gr deletion found in our study may have relevance for the interpretation of case control studies dealing with admixed populations.

P10.23 Prevalence of hereditary deafness in population of 7 rural Districts of Kirov region, Russia.

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The current report analyzes the investigation of prevalence of hereditary deafness forms in 7 districts of Kirov region (Bogorodsk, Nema, Svecha, Sovietsk, Suna, Uni, Shabalino). The total size of the examined population was made 84,048 persons. The study was carried out according to the protocol elaborated in the genetic epidemiology laboratory of Research Centre for Medical Genetics. The protocol allowed to reveal syndromal deafness (not less than 400 hereditary syndromes) and the isolated relative deafness. The examination included ENT physician and ophthalmologist examination, genetic counseling, and DNA-analysis. The DNA diagnosis was made in all cases of non-syndromal deafness and in some cases of syndromal deafness. Different forms of deafness were detected in 624 patients (prevalence of deafness was 1 per 135 persons). Analysis of etiological structure showed that deafness resulting from inflammatory diseases of middle ear and presbyacusis had the following prevalence: 1:316 and 1:466 respectively. Prevalence of non-syndromal deafness with no history of environmental risk factors was 1:506 persons (from 1:921 in Nema to 1:254 in Suna). Prevalence of syndromal deafness was 1:4,002 (from 1:9,751 persons in Svecha to 1:1,331 persons in Uni). In 7 districts, prevalence of mandibulofacial dysostosis was 1:4,000. Examination by direct sequencing of the encoding part of gene TCOF1 revealed previously unknown mutation in exon 23 in the heterozygous state that leads to substitution of aminoacids alanin for glycine in position 1,176 (Ala1176Gly). Thus, the epidemiological approach to investigation of hereditary pathology revealed high prevalence of hereditary deafness in Kirov region.

P10.24 Monogenic hereditary skeletal disorders in Rostov region, Russia

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The prevalence and incidence of the majority of hereditary skeletal disorders (HSD) are remains not studied now, in view of the big diversity of clinical and genetically form. The purpose of the present research was studying of prevalence and genetically heterogeneity of HSD in Rostov region (12 districts). The total size of investigated population was 497460 persons. The research was conducted under the original protocol, providing for detection of more than 2500 various hereditary diseases (OMIM) and syndromes, including all hereditary skeletal disorders. In the results 643 patients from 423 families were detected with different forms of HSD (including isolated forms HSD and the skeletal dysphasia's which a part of various hereditary syndromes are). Value of prevalence of autosomal dominant (AD) and autosomal recessive (AR) forms HSD was paid off on 10000 surveyed population. For the X-linked forms - was paid off on 10000 males. Prevalence of the isolated AD forms of HSD has compounded 5.61 on 10000, AR forms - 0.54 on 10000, X-linked forms - 0.12 on 10000 males. Total value of prevalence (when isolate and syndromes' forms of HSD was include) of AD forms have compounded 10.92 on 10000 with appreciable statistically authentic variation between districts from 5.54 to 17.56. For AR forms the value was amount 1.73 which vary from 0.39 to 3.25, for X-linked forms it was 0.60 with vary from 0 to 2.67. Thus, in the presented research we managed to estimate prevalence of HSD in Rostov region.

P10.25 The occurrence of mutations in HFE gene in Romany population in Slovakia.

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Hereditary hemochromatosis is a genetic disease characterized by accumulating of excessive iron in the organism. Three mutations in the HFE gene are associated with the disease: C282Y, H63D and S65C. The aim of this study was to determine the frequency of HFE mutations in the Romany (Gypsy) population in Slovakia. Romany community in Slovakia is one of the European largest. It represents genetically isolated population with a rate of consanguinity and inbreeding about 10 to 100 time higher than in Slovak (Non-Romany) population. Altogether 212 healthy individuals of Slovak Romany population were analyzed for the presence of HFE mutations. The observed allele frequencies were 3.8%, 4.5% and 0.0% for C282Y, H63D and S65C mutation respectively. We identified 1.4% of H63D/H63D homozygotes, no individual in our sample was C282Y/C282Y homozygous or compound heterozygous. Occurrence of H63D mutation in the Romany population was significantly lower than in the Non-Romany population in Slovakia and it was also lower than the prevalence in the Northern India, where Romanies originate. Probable reason of this result is a founder effect and a high degree of inbreeding among Romanies in Slovakia.

P10.26 Interleukin-23 receptor gene haplotypes in diseases known to associate with individual interleukin-23 receptor gene mutations

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The interleukin-23 receptor gene (*IL23R*) encodes a subunit of the IL23-receptor binding the IL-23 cytokine. IL-23 induces differentiation of naive CD4⁺ T cells into pathogenic T helper cells (Th17/Th_{IL-17}) that produce proinflammatory cytokines and thus play a role in development of autoimmune diseases. Previously we investigated the possible effect of *IL23R* SNPs on the development of psoriasis (n=214 patients) and ankylosing spondylitis (n=206 patients). In psoriasis we observed a significant increase in the carriage of the minor allele of rs11805303 SNP and in the prevalence of the homozygous genotype of rs2201841 and rs10889677 polymorphisms. The results were similar in the ankylosing spondylitis group with the addition of rs1004819 and rs11209026 allele distributions also showing significant differences from those of healthy subjects'. Based on these results we tested the haplotype distributions of *IL23R* SNPs in these two distinct diseases. Haplotype blocks were determined using Haplovview v4.1 according to the International HapMap Project database. Block 1 contained the rs1004819 and rs11805303 SNPs, Block 2 the rs10489629 and rs2201841 SNPs. We found no significant difference between any of the haplotype distributions in psoriasis and ankylosing spondylitis. The fact that two distinct diseases share the same haplotype profile can underline the significance of the IL-23/IL-17 pathway and of the *IL23R* gene in autoimmune diseases, and more importantly indicates the importance of studying the effects of not only individual SNPs but also haplotypes on disease development.

P10.27 Microsatellite allelic imbalance at 14q13.2 indicates an additional susceptibility locus for the Juvenile Idiopathic Arthritis in Latvian population

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Juvenile idiopathic arthritis (JIA) is autoimmune, clinically heterogeneous, chronic rheumatic disease with onset before age 16 and duration at least 6 weeks. Both genetic and environmental risk factors are involved in pathogenesis of the disease. The described JIA susceptibility genes appear to account only for a small part of genetic contribution to the disease, other susceptibility loci involved in metabolic pathways affecting disease pathogenesis should be sought. The 14q locus appears to be prospective from this point of view, as it harbours several genes linked to autoimmune diseases. To identify novel JIA

susceptibility loci, 270 Kb region of chromosome 14q encompassing FAM177A1, KIAA0391 and PSMA6 genes was genotyped in 97 oligo-arthritides (JloA) and 50 polyarthritides (JlpA) patients and 230 healthy individuals by five HSMS markers of 14q13.2 region. Direct sequencing revealed two variable components of (CAA)n(A)m motif in HSMS602 (FAM177A1 gene). Repeat (AC)5AT(AC)n of HSMS701 (KIAA0391 gene) was variable only in its downstream part. Allele (AC)5AT(AC)15 of HSMS701 was found to be in strong association with JIA ($P=4.91\times 10^{-5}$, OR=15.43) and close to modest with JlpA ($P=1.64\times 10^{-3}$, OR=24). Alleles (AC)5AT(AC)18 of HSMS701 and (TG)10 of HSMS702 appear to be JIA and JloA risk factors ($P=1.09\times 10^{-3}$, OR=3.16 and $P=2.00\times 10^{-3}$, OR=7.39 correspondingly), but allele 168bp of HSMS602 ($P=9.02\times 10^{-4}$, OR=0.28) appears to be protective. Two heterozygote genotypes (TG)20/23 of HSMS006 and (AC)22/23 of HSMS801 manifested association with JIA ($P<2\times 10^{-3}$), but homozygote (TG)19/19 was found to be protective ($P=5.41\times 10^{-4}$, OR=0.1). Taken together our results define an additional susceptibility locus for JIA at 14q13.2 genomic region encompassing KIAA0391 and PSMA6 genes.

P10.28 Geographic distribution of the LCE3C-LCE3B deletion, a susceptibility factor for psoriasis, across different human ethnic groups

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Copy number variants (CNVs) contribute to inter-individual genomic and phenotypic variation. Several studies have demonstrated that some CNVs show population differences. Mainly CNVs showing population differences contain genes related to disease or to functions influenced by environment, therefore being potential substrates for natural selection. One example is the 32-kb deletion involving the LCE3C and LCE3B genes (LCE3C-LCE3B_del), which has been significantly associated with risk for psoriasis in European and Asian populations. Since psoriasis is an immune-related skin disease, with a higher prevalence in subjects descending from Northern European countries, it would be expected to find differences on the frequency of LCE3C-LCE3B_del among populations of different geographic origin. Here we provide a comprehensive population genetic analysis of LCE3C-LCE3B_del in populations groups from the Human Genome Diversity Panel (HGDP). We have developed a PCR-based genotyping assay and have analyzed the pattern of linkage disequilibrium (LD) between LCE3C-LCE3B_del and a tagSNP (rs4112788) across the HGDP samples. LCE3C-LCE3B_del was detected in all populations, with allele frequencies ranging between 30% in sub-Saharan Africans to 70% in Native Americans. Strong LD between rs4112788 and LCE3C-LCE3B_del was detected in all HGDP populations with the exception of the sub-Saharan Africans. The strong LD and the wide distribution of LCE3C-LCE3B_del across populations indicate a single origin for this deletion. Moreover, the data suggest that the differences in the population distribution of LCE3C-LCE3B_del are not due to natural selection, but are the consequence of genetic drift produced by a strong bottleneck during the expansion of humans out of Africa.

P10.29** Age and origin of the Swedish Y111C/KCNQ1 founder mutation

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Background: The Y111C/KCNQ1-mutation has been identified as a major cause of the Long QT Syndrome in Sweden. Here we investigate its origin, age and possible founder-nature.

Methods: All identified Swedish Y111C families were genealogically investigated. In 26 index families (2 individuals per family) and 24 healthy Swedish controls haplotype analysis was performed, using 15 satellite markers, 6 upstream and 9 downstream of the KCNQ1 gene (distance ~8 cM). The ESTIAGE computer software was used for estimating the age of the mutation.

Results: We have identified 166 Y111C mutation-carriers in 36 index

families. Their ancestors were traced back to a northern inland region, from where the population spread, migrating along the Ångerman river valley during the 17th-19th century. Twenty-six index cases are genealogical descendants of a founder couple born in 1605/1614.

The 26 haplotyped Y111C families share 3-15 (median 13) uncommon allele-variants surrounding the Y111C locus, with allele-frequencies ranging between 0.02-0.69 (median 0.17) in the healthy controls. Familial haplotypes co-segregate within the subdivisions of the pedigree, supporting the genealogical data.

The estimated age of the mutation is 31 generations (95% CI 23; 41). Assuming that one generation is 25 years, the mutation is 775 years old (95% CI 575; 1,025).

Conclusion: The Swedish Y111C/KCNQ1 founder mutation was probably brought to northern Sweden by settlers in the 13th century. Strong regional founder effects and a mild phenotype probably enabled the enrichment of this mutation in the Swedish population.

The Y111C founder population constitutes an invaluable asset for future studies.

P10.30 Polymorphisms of estrogen metabolism genes and longevity: a follow-up study in the Italian population.

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Aromatase (CYP19) and estrogen receptor-alpha (ESR1) are both involved in estrogen metabolism. Aromatase catalyzes a critical reaction for estrogen biosynthesis from androgens, and estrogen receptor-alpha mediates the biological action of estrogens. Recent findings have revealed that estrogens have a relevant role not only in female but also in male ageing. In the present paper we investigated the possible association of ESR1 (PvuII, rs2234693) and CYP19 (rs4646) polymorphisms with longevity by means of a follow-up study. The study population consisted of 258 individuals (43.3% males) born in 1900-1930, who were living in the district of Salerno (Southern Italy) in the year 2000. The mortality information of these subjects was collected in the year 2009. Using the mortality data the sample was divided into two groups of subjects surviving over 90 years (≥ 90 years) or not. The analysis of ESR1 and CYP19 genotype distribution revealed an excess of homozygotes ESR1 PP (0.30 vs 0.19) and genotypes carrying CYP19 T allele (0.69 vs 0.55) in long-lived subjects compared to non survivors. The logistic regression analysis showed that, after adjusting for sex, both ESR1 and CYP19 genotypes were independently associated with survival over 90 years with OR= 2.20 (C.I. 95% 1.04-4.59, $p = 0.03$) and 1.92 (C.I. 95% 1.04-3.55, $p = 0.03$) respectively. Present findings highlight that estrogen metabolism genes are not only involved in reproduction, but also in ageing and exert a pleiotropic action across the whole human life course.

P10.31 Pigmentation gene MC1R shows strong genetic patterning in Eurasia

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The population genetics of the Central Asian heartland is of great interest, both in and of itself and because prehistoric populations ancestral to those of Europe and East Asia are thought to have passed through this region. However, Central Asia has not been as extensively studied as the eastern and western parts of the continent. Of particular interest in this geographical context is the MC1R gene due to its importance in skin pigmentation. We present a comprehensive analysis of allele/haplotype frequencies from five functional SNPs (rs1805005, rs2228479, rs1805007, rs1805008, and rs885479) in MC1R throughout Eurasia, including from 12,151 individuals from 141 regional populations, focusing on novel genotype data from 38 Central Asian populations.

We performed analyses of allelic differentiation (F_{ST}) and direct statistical tests of allele/haplotype frequency differences using Fisher's exact test. These revealed several significant differences between popula-

tions for all SNPs, and a striking pattern of highly significant differences between even some Central Asian populations (e.g. $p=2.38\times10^{-23}$ for Iranians from Samarkand and neighbouring Kyrgyz).

Our results confirm divergent trends in eastern and western Eurasia, with strong minor allele frequency gradients, perhaps reflecting selection of different variants in *MC1R*, such as are seen in other pigmentation genes. Data from the mitochondrial genome and Y chromosome are included to provide a framework for interpretation. Finally, as Central Asia consists of a patchwork of linguistic areas with related language groups frequently being geographically distant from one another, we investigate whether any subordinate genetic patterning is associated with linguistic rather than geographic affiliation.

P10.32 The Investigation af C1236t and C3435t Polymorphisms In MDR1 (ABCB1) Gene In Familial Mediterranean Fever (FMF) Patients

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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. Up to date more than 50 SNPs have been described in the *MDR1* gene .

FMF is considered as an autosomal recessive hereditary disease, associated with a single gene named MEFV. However, about one-third of FMF patients bear a single mutation on one allele, suggesting that the disease might be transferred as an autosomal dominant trait with partial penetration.

The aim of this study was to performe a genotyping and haplotyping analysis of *MDR1* gene in FMF patients. Two MDR-1 genetic markers (C1236T and C3435T) were analyzed in 142 FMF patients and 107 unrelated Turkish subjects. All subjects were genotyped by PCR-restriction fragment length polymorphism (RFLP) analysis, and Arlequin Software was utilized to estimate the genotype and haplotype frequencies, and SPSS 16.0 Software was utilized to estimate OR and Chi-square tests.

In FMF patients showed higher frequency of the 3435 CT genotype compared with the control group (0.60 vs. 0.41; $P = 0.003$; odds ratio, [OR], 2.14; 95% confidence interval [95% CI], 1.28-3.56) (Table 1). For the CC-CT (1236-3435) genotype frequency dedected significantly higher in FMF patients compared with the control group (0.13 vs. 0.04; $P<0.01$; OR, 3.83; 95% CI, 1.26-11.68).

| Table 1 Compared allelic and genotyping frequencies of <i>MDR1</i> gene loci with FMF patients and control | | | | | | | | | | | | | |
|--|------|-------------------|--------|-------------|----------|-----------------|----------|----------|----------|-----------|---------------|---------------------|----------|
| Position | Exon | Amino acid change | Allele | FMF (N=284) | | Control (N=214) | | <i>P</i> | Genotype | FMF (142) | Control (107) | Odds Ratio (95% CI) | <i>P</i> |
| | | | | <i>n</i> | <i>F</i> | <i>n</i> | <i>F</i> | | | | | | |
| 1236 | 12 | G412G | C | 152 | 0.535 | 98 | 0.458 | 0.053 | CC | 42 | 21 | 1.72 (0.95-3.13) | 0.050 |
| | | | | | | | | | CT | 68 | 55 | 0.84 (0.51-1.38) | 0.285 |
| | | | | 132 | 0.465 | 116 | 0.542 | | TT | 32 | 31 | 0.75 (0.42-1.33) | 0.199 |
| | | I1145I | C | 133 | 0.468 | 102 | 0.477 | 0.463 | CC | 24 | 29 | 0.55 (0.30-1.01) | 0.037 |
| | | | | | | | | | CT | 85 | 44 | 2.14* (1.28-3.56) | 0.003* |
| | | | | 151 | 0.532 | 112 | 0.523 | | TT | 33 | 34 | 0.65 (0.37-1.14) | 0.087 |

P10.33 Haplotype profile of multidrug resistance 1 (MDR1/ABCB1) gene in the average Hungarian and Roma population samples

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The human multidrug resistance gene (MDR1/ABCB1) is an energy-dependent drug-transport pump, plays an important role in the bio-

availability of a wide variety of drugs and responsible for the multidrug resistance in cancer cells. The aim of this study was to investigate the genetic variability, haplotype profile and ethnic differences of the MDR1 polymorphisms in healthy Roma and Hungarian population samples. Therefore, 503 Hungarian and 465 Roma healthy subjects were genotyped for the C1236T (rs1128503), G2677T/A (rs2032582, Ala893Thr/Ser) and C3435T (rs1045642) variants by PCR-RFLP assay. Differences were found in the MDR1 1236 CC (20.7 vs. 33.2%) and TT genotypes (30.8 vs. 21.9%), in T allele frequency (0.551 vs. 0.443) ($p<0.002$), and in 3435T allele frequency (0.482 vs. 0.527, $p<0.04$) between the two studied groups. Futhermore, the frequency of CGC, CGT, CTT haplotypes was significantly higher in Hungarian population than in Roma (41.4 vs. 35.3%, 9.04 vs. 6.02%, 2.88 vs. 1.08%, respectively; $p<0.009$), whereas the frequency of TGC and TTC haplotypes was higher in Roma population than in the Hungarian (7.31 vs. 1.68%, 6.67 vs. 2.08%, respectively; $p<0.001$). The results of MDR1 polymorphisms found in the Hungarian population were similar to that observed in other Caucasian populations, however, some differences were observed in the haplotype structure. By contrast, the Roma population differs from Hungarians, from Caucasians, and also from populations reported so far from India in the incidence of MDR1 common variants and haplotypes. Observed interethnic differences can have consequences for choice of treatment.

P10.34 Molecular epidemiology analysis of the most common mtDNA mutations in Hungary

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Background: Many heteroplasmic point mutations in the mitochondrial genome (mtDNA) have been associated with a wide scale of neurological symptoms. We report a prospective 10-year molecular study of mitochondrial DNA mutations in Hungarian patients with highly suggestive of mitochondrial diseases.

Methods: The mutations frequencies of the most common mtDNA mutations (A3243G, A8344G, A8356G, T8993C, T8993G, G3460A, G11778A, T14484C) were investigated in patients with maternal sensoneurial hearing loss, stroke-like episodes, ataxia, epilepsy and myopathy with undetermined etiology. We screened 631 Hungarian patients in North-East, South-West and Central Hungary for this mutation. The mtDNA analysis was performed mostly from blood. If muscle tissue was available, the genetic analysis was performed from muscle also. Each substitution were tested by PCR-RFLP methodology with different restriction enzymes.

Results: Of the 631 patients screened for MELAS A3243G, 2.22% were positive for the mutation, whereas for MERRF A8344G, 2.53% carried the mutation and for LS/NARP T8993C, 0.32% and T8993G, 0.16% carried the mutation. The outcomes for the LHON mutations were and G3460A, 0%, G11778A, 0.32%, T14484C, 0%. In 1 patients the mutation was present only in the postmitotic muscle tissue.

Conclusion: The incidence of the most common mtDNA pathogenic point mutations was relatively low in the investigated cohort. The mutation frequency of the analysed substitutions was 4.9% in Hungarian patients with suggestive of mitochondrial disorders. Our results suggest that screening the most common alterations of the mitochondrial genome is not sufficient to clarify the molecular background of the mitochondrial disorders.

P10.35 Inferences of maternal genetic heritage of Bayash Roma in Croatia

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The Bayash are a branch of Romanian speaking Roma living dispersedly in Central, Eastern and Southeastern Europe. In order to investigate the origin of the Croatian Bayash maternal gene pool, we have sequenced hyper variable segment I (HVS-I) of mitochondrial

DNA control region and typed relevant RFLP sites in the mitochondrial DNA of 390 Bayash from two Croatian regions: Baranja (235) and Medjimurje (155). The most frequent maternal lineages of the Bayash in Croatia (M5a and M35) belong to the M cluster testifying about their ancient Indian origin. European admixture in Bayash maternal gene pool is most evident in the occurrence of haplogroup H lineages (H1, H2, H4, H5, H6 and H11) with a frequency of 26%. Characteristic of Croatian Bayash maternal lineages is a significantly higher frequency of haplogroup X2 (19.5%) than in other Roma populations; these lineages harbour specific HVS-I mutations not present in any of the populations that normally have this haplogroup.

Haplogroups and haplotype distribution clearly shows that Bayash from Medjimurje represent isolated subset that shares common origin with Bayash from Baranja. Obtained results suggest that today's Roma and Bayash genetic structure was influenced by endogamy and isolation along with genetic drift in small and dispersed subpopulations and few episodes of gene flow.

P10.36 Mitochondrial DNA Mutation Associated Leber Hereditary Optic Neuropathy: Prevalence Rate Reflected By Positive Laboratory Tests In Hungary

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Leber hereditary optic neuropathy (LHON) is a maternally inherited early-onset acute or subacute central vision loss. Mitochondrial mutations especially in the subunits of NADH dehydrogenase and cytochrome B encoding genes have been found to associate with the disease. Here we analyzed the profile of test results of the most common mitochondrial mutations (primary: G3460A, G11778A, G14459A, T14484C, G15257C; secondary: C3275A, G3316A, T3394C, T4216C, G7444A, T9101C, G13708A), which variants were analyzed using PCR/RFLP assays and direct sequencing of suspected patients' DNA. A total of 126 patients with LHON resembling phenotype (62 males and 64 females), who were sent to genetic tests between 1999 and 2009, were involved in the study. Positive test results were found in 31.3% of the males and 26.6% of the females for primary LHON mutations. The 11778A was the most frequent primary LHON mutation both in males and females (n=14, 11.1% and n=11, 8.73% of total patients, respectively). The 3460A was present in one male and five females (3.97% of total patients); while the 15257C variant was found in four males (3.17% of total patients), and one female. Surprisingly, the 14484C variant, which is one of the most common primary LHON mutations, was found in only one patient. Besides, only the T4216C and G13708A of secondary LHON mutations were detected in our patients (n=16, 12.7%, n=10, 7.93% in males; n=18, 14.3%, n=13, 10.3% in females). Our results suggest that the mutation profile of the disease in Hungary differs from that reported from other European populations.

P10.37 Mitochondrial DNA diversity in a coastal population from Tunisia

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Situated between Europe, the Middle East and Sub-Saharan Africa, Tunisia represents a crossroads for human migrations. Studies describing the maternal lineage of Tunisian population have so far been conducted mainly on Southern and Northern Tunisia, among Berber and on few non Berber populations. With the aim to enrich the Tunisian mtDNA reference database we analysed for the first time the mitochondrial DNA variability in the population of Monastir, a coastal city from central Tunisia.

The hypervariable region HVS1 was sequenced and RFLP analyses were carried out in a sample of 52 individuals from Monastir. 46 different mtDNA haplotypes were identified and assigned to 12 haplogroups which showed a high heterogeneous gene pool. The main haplogroups are U (19.2%), H (15.4%), L3 (13.5%) and T (9.6%). The Eurasian haplogroups were predominant (63%) followed by sub-Saharan L lineages (23.5%) and specific North African clades: U6 and M1 (13.5%).

Compared to previous studies on Tunisian populations this is the first time that haplogroup U was among the main haplogroup. In addition,

the level of U5 in our study was higher than that found in previous studies occurred in Tunisia.

P10.38 Haplotypic structure of the mitochondrial DNA polymerase gamma (POLG) gene

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Variation of mitochondrial DNA polymerase gamma (POLG) gene has been extensively studied in health and disease because it is a genetic locus for several mitochondrial diseases with over 150 mutations currently identified (Copeland 2010). However little is known about the haplotypic structure of this gene. Here we have sequenced its intron 2 and analyzed distribution of the POLG gene haplotypes in two North Eurasian populations - in Russians (n=63) and Buryats (n=90). Five polymorphic loci - three previously described rs2283430 (G/T), rs2239286 (G/C) and rs2247233 (G/A) and two novel polymorphisms - were revealed. Among novel polymorphisms, first of them was a transition G>A, which is located in position +14 in relation to rs2283430, and second one was a transition A>G at position -5 in relation to rs2239286. In total, five haplotypes comprising two haplogroups were revealed. Haplotype A consists of haplotype TGACA and haplotype B is represented by haplotypes GGAGG, GGAGA, GAAGG and GGGGG (for loci rs2283430, +14 from rs2283430, -5 from rs2239286, rs2239286 and rs2247233, respectively). Interestingly that previously detected in Russian population mutations in exon 3 were found on different backgrounds - G268A on haplotype A and T251I on haplotype B. This work was supported by the grant from the Far-East Branch of the Russian Academy of Sciences (09-3-A-06-221).

P10.39 Genetic variation in Bulgarians: a mitochondrial DNA perspective

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The structure and diversity of the Bulgarian mitochondrial DNA (mtDNA) gene pool is still almost unknown. In the present study, we have evaluated the extent and nature of mtDNA variation in the largest Bulgarian sample to date, comprising 855 healthy unrelated subjects from across the country.

The analysis was performed by sequencing about 850 base pairs (from np 16000 to np 250) of the mtDNA control region, followed by the hierarchical RFLP survey of numerous diagnostic coding-region markers. Overall, this approach allowed the identification of 586 different haplotypes and their classification into 79 known haplogroups or paragroups.

The observed pattern of mtDNA diversity in Bulgarians is mainly shaped by haplogroups (H and U) dated to the Upper Paleolithic period. The spread of majority of the subclades of these haplogroups is related with waves of post-LGM recolonization. A fraction of the Bulgarian mtDNA gene pool is allocated to haplogroups, which represent Neolithic genetic component.

In the comparisons of the observed haplogroup frequencies with those from a wide range of western Eurasian populations, Bulgarians do not group with the great majority of other Europeans and differ substantially from Near Eastern populations. This reflects the peculiarity of the Bulgarian mtDNA gene pool, determined by its history and demographic processes.

P10.40 Analysis of human mtDNA mutational spectrum revealed by phylogenetic reconstruction

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The mitochondrial genome has been the most widely used system for the investigation of the evolutionary history of our species. It has been the system of choice because of its high rate of sequence divergence

and its uniparental, maternal inheritance. Advent of human population genomics has lead to rapid accumulation of complete mtDNA sequences. At present there are more than 6500 human mitochondrial genomes available in GenBank. This report is based on the study of mutational spectrum revealed in phylogenetic tree of all available complete genomes. The maximum parsimony tree was reconstructed with the mtPhyl package (<http://eltsov.org/mtphyl.aspx>). The relationship between mutation frequency throughout the tree and its conservation index was studied as well as dependence of aminoacid change position in the tree and its physicochemical properties. Equation which allows estimating the mutational saturability was deduced with which it was shown that approximately 135 000 complete sequences necessary to reach 90% saturation. The analysis revealed mutational hot spots and showed that approximately 14% of human mitochondrial genomes deposited in GenBank contain errors.

P10.41 Mitochondrial genome diversity in Ulchi, the tungusic-speaking tribe of the Russian Far East

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The present report is based on the study of mtDNA variation in Ulchi (n=74), a Tungusic-speaking tribe of hunters and fishermen dispersed along the lakes and reaches of the Lower Amur. MtDNA analysis revealed 39 distinct mtDNA haplotypes belonging to 21 Eurasian haplogroups C2-C3, D3-D8, D11, G1-G2, M7-M9, Z, B, F, N9, Y and U4, with overall N macrohaplogroup derivatives frequency 53%, M - 43%, and R - 4%. It is generally accepted, that rapid migration along the Asian Pacific margin brought undifferentiated M as far as Japan and Russian Far East. The regionally differentiated MD, MG and M7 mtDNA lineages were of our special interest in light of the Late Pleistocene migrations of modern humans into circum-Pacific region. One of our major findings is the discovery of M7a2 haplotype with 16140-16187-16209-16223-16519 HVSI motif which is specific for ancient Okhotsk people (Sato et al. 2007). Ancient admixture of East and West Eurasians is also found in the Ulchi population of the Lower Amur region. The presence of such haplogroups as N9 and Y in south-eastern Siberia, with 50% frequency of Y in Ulchi, reflects complex genetic and demographic history, including natural selection and founder effects. The coalescent dates and spatial distribution of N9 and Y across Eurasia let us to suggest that the root mutation 5417 emerged somewhere in Southwestern Asia ~40.0 ka, and its particular derivatives (N9a, N9b, Y) have been involved in the eastward expansion, with the Y lineage (~23 ka) being the most successful at the periphery.

P10.42 Effect of the C677T-MTHFR genotype on metabolic factors of endothelial dysfunction: homocysteine concentrations, von Willebrand factor plasma levels and Ca²⁺ concentrations in patients with Chronic Heart Failure (CHF) who lived in Blockaded Leningrad during WWII, St. Petersburg, Russia.

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Introduction: Chronic heart failure (CHF) is a major health problem for people, who lived in blockaded Leningrad (bL) during World War II. We have the unique opportunity to investigate remote influence of the prolonged starvation in childhood on development of CHF in senility. We have investigate the associations of the C677T-MTHFR genotype on metabolic factors of endothelial dysfunction: plasma homocysteine concentrations, von Willebrand factor plasma levels and Ca²⁺ concentrations.

Materials: 489 patients with CHF from bL (420 females and 69 males, age 68-88 years), 156 individuals with CHF from other places, 85 women after 80 years without CHF.

Genotypes were determined by PCR-RPLF.

Results: There were no differences in genotypes distributions of MTHFR C677T among our groups. In group of patients with severe CHF (75 females, 15 males, age 73±1,8 years) plasma homocyste-

ine concentrations were not significant higher in T/T cariers (18, 94 ± 2,91 mkmol/l, 14,87± 0,84 mkmol/l, p= 0,11). Significantly higher von Willebrand factor plasma levels were in T/T patients compared to C/C (238,57±29,29 ; 177,81±10,05, p= 0,04) and C/T carriers (238,57±29,29, 176,88±12,68, p= 0,04). C/T patients had higher Ca²⁺ concentrations compared to C/C+ T/T (1,23±0,03 mmol/l, 1,15±0,02 mmol/l , p=0,01).

Conclusion: T allele of MTHFR gene is risk factor for CHF in this group of patients. We suggest that DNA-tasting of C677T-MTHFR genotype is important for the prognosis and following drug treatment of such patients.

P10.43 Mutation rates of 15 short tandem repeat loci used in paternity testing and forensic analysis

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Knowledge about mutation rates of short-tandem-repeat (STR) or microsatellite loci used in paternity testing and forensic analysis is crucial for the correct interpretation of resulting genetic profiles. The aim of this study was to determine the type and frequency of germline mutations in 15 STR loci. A total of 100 paternity cases were analyzed using AmpFISTR Identifier kit. The parenthood in each of these cases was highly validated (probability>99.99%). We identified 7 mutations in 6 different loci (D21S11, D19S433, D16S539, D2S1358, FGA and CSF1PO) with locus specific mutation rate varying between 0 and 1x10⁻², and overall average mutation rate estimated at 2.3x10⁻³. Five mutations occurred in the male germline while two mutations could not be distinguished. All seven mutations were single repeat changes, with 5 (71.4%) single repeat gains and 2 (28.6%) single repeat losses. In one paternity case with mutation at D16S539 locus, analysis of Y-haplotype using AmpFISTR Y-filer kit revealed additional mutation at DYS458 locus. The mutation event is very crucial for forensic DNA testing and accumulation of STR mutation data is extremely important for genetic profile interpretation.

P10.44** Gene variants from lipid-related pathways and risk for myocardial infarction: Results from two community-based longitudinal studies

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Background: Lipoprotein levels in blood and several familial lipid metabolism disorders are closely associated with initiation and progression of atherosclerosis, and incidence of myocardial infarction (MI). Therefore, we hypothesized that variants in genes associated with circulating lipid levels also would be associated with MI.

Methods: Using age- and sex-adjusted additive genetic models, we analyzed 629 single nucleotide polymorphisms (SNPs) spanning 59 candidate gene regions proposed to be involved in lipid-related pathways in relation to incidence of MI in 2,523 participants of the Swedish Twin Register (STR; 57% women). All associations with nominal P<0.01 were further investigated in the Uppsala Longitudinal Study of Adult Men (ULSAM; N=1,095) by *in silico* replication supplemented with additional *de novo* genotyping.

Results: There were 14 SNP-MI associations in STR with nominal P<0.01 that we attempted to replicate in ULSAM. Of these, data already available from the ABCA1 locus (rs4149313 in STR; rs7031748 in ULSAM [r²=1]) showed evidence of replication in ULSAM; the HDL-lowering allele was associated with a substantially higher MI risk (hazard ratio, 1.34 and 1.42; 95% confidence interval, 1.09-1.65 and 1.08-1.87; P, 0.005 and 0.013 in STR and ULSAM, respectively). Direct genotyping for several other loci is still ongoing.

Conclusions: In two community-based samples, we observed several potentially interesting associations between lipid-related SNPs and MI incidence. Of these, we could confirm the association between the ABCA1 locus and MI across both studies. However, several other associations in our preliminary analysis in STR could represent true findings, and replication genotyping is currently ongoing.

P10.45** A genome-wide linkage scan of personality traits reveals new loci

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The Neuroticism-Extraversion-Openness Five Factor Inventory (NEO-FFI) categorizes human personality into five broad dimensions Neuroticism, Extraversion, Openness, Conscientiousness and Agreeableness. These traits are highly heritable (20-60%) and have proven to be strong predictors of not only several psychiatric and non-psychiatric disorders but also to social behavior and work performance. Within the Erasmus Rucphen Family study (ERF), we phenotyped 2368 related individuals with the NEO personality inventory. Affected-only genome-wide linkage analysis was performed using the Illumina 6K linkage panel for a set of highest scorers (> 90 th percentile, $n \sim 200$) for each of the five traits. Multipoint parametric linkage analysis assuming dominant and recessive models and non parametric linkage analysis was performed. Significant evidence of linkage to chromosome 20p ($LOD = 5.86$) was observed for conscientiousness, a trait strongly related to job performance. Additionally suggestive evidence of linkage was observed for Neuroticism at 19q ($LOD = 3.73$), 21q ($LOD = 3.42$) and 22q ($LOD = 3.07$); Extraversion at chromosomes 1p ($LOD = 3.05$), 9q ($LOD = 3$), and 12q ($LOD = 4.01$); Openness at 12q ($LOD = 3.71$) and 19q ($LOD = 3.0$) and Agreeableness at 2p ($LOD = 3.11$), 6q ($LOD = 3.54$), 17q ($LOD = 3.32$) and 21q ($LOD = 3.64$). For conscientiousness at 20p we identified two distinct haplotypes segregating in 6 families. The region overlaps with one identified earlier for cognitive function in the same population. These findings suggest that we have identified a new locus involved in an important personality trait, which may have a wider impact.

P10.46 Association between Cystatin C polymorphism and corpulence

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Background: Cystatin C expression rate in adipose tissue and circulating levels in blood are two to threefold higher in obese subjects than in lean controls.

Work hypothesis: Our hypothesis is that common variants in Cystatin C gene could play a role in the evolution of BMI during lifetime.

Methods: Tag SNPs were selected to obtain a full coverage of Cystatin C coding sequence based on hapmap data. Tag SNPs were genotyped in population based cohorts: 4300 subjects from the SUVIMAX study ($BMI=23.78 \pm 0.05 \text{ kg/m}^2$, $age=49.58 \pm 0.10 \text{ yrs}$), 750 women from the SPAWN cohort in Sweden ($BMI= 21.73 \pm 0.10 \text{ kg/m}^2$, $age=29.16 \pm 0.16 \text{ yrs}$) and 1500 men from Denmark ($BMI=27.00 \pm 0.16 \text{ kg/m}^2$, $age=19.92 \pm 0.05 \text{ yrs}$). Association between BMI evolution and genotypes was carried out with a linear mixed model.

Results: One SNP, rs2424577, was significantly associated with differences in BMI over time in all three groups. G/G carriers had a significantly lower BMI over time ($p<0.05$) in the SUVIMAX group and in the subgroup of Danish men who were lean at inclusion ($BMI<30 \text{ kg/m}^2$) than the carriers of the two other genotypes (A/G and A/A). Surprisingly, in the SPAWN cohort, G/G carriers had a significantly higher BMI than the carriers of the other genotypes. These observations may be due to the effect of a specific haplotype, since no such difference was found between males and females in the SUVIMAX group.

Conclusions and perspectives: These results bring insights into the contribution of Cystatin C in time dependant corpulence. Haplotype analyses are ongoing in all 3 groups.

P10.47 The P53 gene polymorphisms and life and family history of cancer in Croatian elderly population

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The P53 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 retrospective study we investigated the association of two widely investigated polymorphisms of P53 gene with life and family history of cancer in a sample of Croatian elderly population. We studied the Arg72Pro and PIN3 (+16bp) polymorphisms in 124 persons aged 85-101 yrs. The allele frequencies in senescent sample were similar to those reported for general population of Croatia: 77.3% vs. 75.9% (for Arg) and 86.6% vs. 85.7% (for A1). On total sample level no sex or age differences were found in life and family history variables or in genotype distribution. However, when two age groups were formed - "younger" (85-90 yrs; $N=58$) and "older" (91-101 yrs; $N=66$) - new relations emerged. In contrast to older subjects showing homogeneity for tumor occurrence across genotypes in both polymorphisms, in younger subjects Arg allele as well as ArgArg genotype of the Arg72Pro polymorphisms was consistently more frequently recorded in persons with life and family history of cancer. As it was frequently found for younger persons the present preliminary study suggests that Arg allele carry increased risk for cancer in aged persons as well. However, it seems that in very old olds (91+ in the present study) the importance of Arg72Pro polymorphism for carcinogenesis diminishes. Further studies considering more narrowly defined pathological substrate are needed to disentangle the reasons for obtained age-related differences.

P10.48 Polymorphisms with relevant role in platelet function in Portuguese population

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Genetic and environmental factors can be responsible of hyper or hypo- reactivity of platelets. We know that more than 30% of natural variation of platelet reactivity seems to be related with genetic factors. The main platelet glycoproteins involved in primary hemostasis are GPIb-V-IX, GPIa-IIa and GPIb-IIIa. Endothelial nitric oxide synthase (eNOS) is also relevant in platelet function. Polymorphisms of these platelet proteins and endothelial eNOS can induce functional platelet alterations. Differences in platelet reactivity and activation among individuals can influence normal hemostasis, presence of thrombosis and response to antithrombotic therapy.

The purpose of this work is to determine allelic and genotypic frequencies of polymorphisms that affect platelet function, namely Kozak, VNTR and HPA-2 polymorphisms of GP1BA gene (GPIb alfa), P1A of ITGB3 gene (GPIIIa), C807T of ITGA2 gene (GPIa), Glu298Asp and T-786C polymorphisms of NO33 gene in a control Portuguese population. Polymorphisms were studied by RFLP methodology.

For the seven polymorphisms studied there are no previous data for Portuguese population. Our results show that allelic and genotypic frequencies in Portuguese population do not differ from the ones found for other Caucasian populations. All genotypes are in Hardy-Weinberg equilibrium.

The genetic characterization of platelet variants in Portuguese population obtained in this work will be important for further studies focused on the role of these molecular markers in specific populations and their possible clinical implications.

P10.49 Analysis of two polymorphisms in genes suspected to take part in Crohn's disease among Polish patients

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Crohn's disease (CD) and ulcerative colitis (UC) belongs to Inflammatory Bowel Diseases (IBD). The etiology of the disease remains unidentified, but it is known, that it is characterized by multifactorial and polygenic background. In the last few years many genes were defined, among others *ATG16L1* as well as *IL23R*, which may predispose for the disease. Up till now, in Polish populations these genes were not studied. The aim of the research was the analysis of polymorphisms: rs2241880 in *ATG16L1* gene and rs7517847 in *IL23R* gene. 156 Polish patients with CD were qualified for this study. Polymorphism analysis was performed in *ATG16L1* rs2241880 gene and rs7517847 in *IL23R* gene using pyrosequencing. The results were compared with an adequately chosen population group. In *ATG16L1* gene the heterozygotic variant TC occurred in 47,9% of patients and did not show significant difference as compared to the population group (47,4%). Homozygotic CC variants occurred among the patients in 22,9% (population 30,8%), however TT genotype occurred in 29,2% among patients and 21,8% in healthy population. In *IL23R* gene the heterozygotic form GT occurred most often (53,7% in patients and 48,7% in population), and GG genotype appeared in 11,6% patients and in 11% of healthy individuals. In turn the TT genotype was observed in 34,7% CD patients and 43% of people not afflicted with the disease. Predominant in both genes were heterozygotic variants. In the vicinity of rs2241880 and rs7517847 no significant changes in the frequency of occurrence of particular genotypes were observed, although in other populations these polymorphisms were related to IBD.

P10.50 Population study at six supplementary STR loci in the representative sample of multinational Bosnia and Herzegovina residents

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In our previous population studies of B&H human population, we have used 17 STR loci included in the *PowerPlex 16[®] System* and *AmpFISTR[®]Identifiler[®]*, twelve Y-chromosomal short tandem repeats loci incorporated in the *PowerPlex[®] Y System*, 14 X-STR loci, as well as 28 Y-chromosome NRY bi-allelic markers. All obtained results were included in Bosnian referent database. In order of future development of this database we have decided to analyze 6 additional STR loci (D22S1045, D1S1656, D10S1248, D2S441, D12S391, SE33). Therefore, we have tested 150 unrelated healthy individuals born in the Bosnia and Herzegovina, from all three main ethnical groups. Qiagen DnaeasyTM Tissue Kit was used for DNA extraction from buccal swabs and blood stains, Quantifiler[®] assay for quantification and *PowerPlex ESI 17[®] System* for amplification and detection. Amplification was carried out as described previously. The total volume of each reaction was 25 μ l. The PCR amplifications have been carried out in PE Gene Amp PCR System Thermal Cycler according to the manufacturer's recommendations. Electrophoresis of the amplification products was preformed on an ABI PRISM 310. Numerical allele designations of the profiles were obtained by processing with *Gene Mappe[®] IDv 3.2 Software* Deviation from Hardy-Weinberg equilibrium, observed and expected heterozygosity, power of discrimination and power of exclusion were calculated. Also, we have compared B&H data with data obtained from other European populations with available data for observed loci. Results of this study are going to be used as guidelines in additional investigation of B&H human population genetic structure.

P10.51 Peroxiredoxin 5 gene polymorphism in the population of Saint-Petersburg

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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, the accumulation of oxidative damages as a result of ROS attack is probably a reason of the aging and age-related pa-

thology. Thus *PRDX5* gene polymorphism may play a role in human longevity. A single nucleotide polymorphism (SNP) in the 5'-flanking regulatory region (c.-540A>C) of the *PRDX5* gene may cause a different expression level of peroxiredoxin 5.

The aims of this study were to compare allele and genotype frequency distribution in two groups of unrelated healthy persons: young (4-17 years old, n=113) and elderly (over 80 years old, n=117, including long-livers: 90-106 years old, n=65) and to compare allele frequencies between St. Petersburg population and another European population. Genotyping was carried out by PCR-RFLP-analysis method. The frequencies distributions were compared by using Chi-square test.

Results: the frequencies of A and C alleles are 63,7% vs 65,4%, 36,3% vs 34,6%; the frequencies of AA, AC and CC genotypes are 38,9% vs 45,3%, 49,6% vs 40,2%, 11,5% vs 14,5% in young and elderly groups, respectively. The genotype distributions are consistent with Hardy-Weinberg equilibrium in both groups. No statistically significant differences in allele and genotype frequency distributions between groups were found. There were also no significant differences in allele frequencies between population of St. Petersburg and European population (KYUGEN_CAU200) reported in the NCBI dbSNP database (n=200, allele frequencies: A-61,2%, C-38,8%).

P10.52 Population genetic analysis of four microsatellite marker linked to retinoblastoma gene

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The most common ocular cancer in children is retinoblastoma. Annual incidence in Iran is approximately 100 new cases. Conclusive identification of RB1 mutations in retinoblastoma is believed to improve the clinical management of affected children and their relatives. However, despite clear clinical benefits, RB1 screening remains difficult, most of the alterations being unique and randomly distributed throughout the entire coding sequence of the huge RB1 gene. In some cases mutation screening of RB1 gene may be time consuming and not beneficial for families that have positive history of retinoblastoma and are planning for a new pregnancy. In such cases linkage analysis using polymorphic microsatellite markers may be the solution. In this study we are evaluating the heterozygosity of four microsatellite markers (D13S128, Rb12, Rb1.20, D13S156) that are either intragenic or closely linked to RB1 gene. Peripheral blood samples of 100 normal Iranian people were collected on EDTA tubes and DNA was extracted from 5-ml whole blood sample using a standard protocol. PCR reactions were fulfilled for each marker and the PCR products were separated using 12% polyacrylamide gel electrophoresis. The Heterozygosity of the above markers is 93%, 69%, 78% and 85% respectively. Our results show that these markers can be used for genetic linkage analysis in Iranian families with retinoblastoma.

P10.53 Transcription of polymorphic human retrotransposon insertions negatively regulates candidate tumor suppressor genes

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Transposons comprise a significant fraction of mammalian genomes, the function of which only gradually emerges. They are silenced, both in the germline and in somatic cells to avoid the detrimental effects of unrestrained transposition. It is therefore unclear at present, whether transposons escaping repression could exert functions that depend on their autonomous transcriptional activity in somatic cells. To address this, we studied two polymorphic intragenic retrotransposon insertions, Ya5-MLS41 (Alu-SINE retrotransposon in *FOG2*) and LTR5_Hs (LTR retrotransposon in *PSD3*) that could be present or absent in human genomes. The allele with the presence of the Alu has a frequency of 0,4, while that of the LTR is 0,2 in European chromosomes. Normalized gene and retrotransposon expression was analyzed with quantitative PCR in two cell types of ~200 individuals. For these two candidate breast cancer suppressor genes, we found retrotransposon transcription to negatively regulate cellular gene expression. This provides for the first time evidence for polymorphic variation in gene-regulatory retrotransposon transcription. These findings also imply that such regula-

tory retrotransposons become candidate tumor markers and potentially novel therapeutic targets. Thus, presence or absence of retrotransposons could be the causative variation of certain cis-eQTLs.

P10.54 Genetic polymorphisms selective value on pre- and postnatal stages of human development

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Selective value of putatively functional polymorphisms of several candidate genes for common diseases was the matter of this study. The groups of chromosomally normal spontaneous abortions (5-12 weeks), middle aged subjects (28-55 years old) and nonagenarians (89-100 years old) were collected in the Tomsk population (288 individuals in total). DNA was genotyped for 19 polymorphisms in 13 genes: MTHFR rs1801133:C>T, ACE rs4343:A>G, AGTR1 rs5186: A>C, GNB3 rs5443:C>T, NOS3 rs2070744:T>C, rs1799983:G>T and VNTR, ADRB2 rs1042713:A>G and rs1042714:C>G, LTA rs909253: A>G, TNFA rs1800629:G>A, IL4 rs2243291:G>C, IL4RA rs1801275: A>G and rs2074570:A>G, IL12A rs568408:G>A, IL12B rs3212227: A>C and rs3212220:G>T, IL12RB1 rs3746190:C>T and rs11575926: G>A. There was redistribution of alleles and genotypes of polymorphisms of genes TNFA and IL12B in the analyzed groups. The IL12B rs3212227 AC/CC genotypes (48% vs 35%; p=0.04) and C allele (28% vs 20%, p=0.04) were registered more often among spontaneous abortions to compare with combined group of middle aged adults and nonagenarians. The TNFA rs1800629 GA/AA genotypes (25% vs 17%, p=0.04) and A allele (14% vs 8%, p=0.04) predominated among living population to compare with spontaneous abortions. In conclusion, the current study provided new facts about different selective significance of polymorphisms of genes TNFA and IL12B on the pre- and postnatal stages of human development.

P10.55 Additional evidence for the absence of pathogenic significance of mutation T1095C (mitochondrial 12S rRNA) in deafness

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Mutations in the mtDNA may contribute to sensorineural hearing loss (SNHL) either in isolation or as a part of multisystem disorder. To date, several mutations in mitochondrial 12S rRNA gene have been found in association with both aminoglycoside-induced and isolated SNHL. Pathogenic significance was confirmed only for A1555G and C1494T mutations, and remains unclear for other mutations (MITOMAP). The T1095C mutation has also been shown to be associated with SNHL in some families mainly of Chinese origin. We performed mtDNA mutation analysis in pedigrees of different ethnic origin with nonsyndromic SNHL and in indigenous Altaians (N=230) in the Altai Republic (South Siberia, Russia). Mutation T1095C was revealed in one Altai patient with profound congenital deafness and in one unrelated control of Altai ethnicity. We also performed the phylogenetic analysis of both mtDNAs with T1095C. The RFLP haplotype and HVS-1/HVS-2 sequence data revealed that both mtDNAs belong to East-Asian mtDNA haplogroup M11. The T1095C is a basal polymorphism of this haplogroup which has been found earlier in the Altai population with frequency 2.2% (Derenko et al., 2007) and among some other East-Asian populations. Based on all these facts, we suggest that the T1095C and deafness in that patient are rather a coincidence than an association. This case provides additional evidence for the absence of pathogenic significance of the T1095C mutation in deafness. This study indicates an importance of mtDNA phylogenetic analysis in mtDNA medical studies in order to avoid erroneous diagnosis and to search for immediate causes of disease.

P10.56 Analysis of nine Y-chromosome STRs (YSTRs) in two Iranian (Kurds and Lurs) ethnic groups

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We studied nine Y-chromosome short tandem repeat (STR) including DYS19, DYS385 ab, DYS389 I/II, DYS390, DYS391, DYS392 and DYS393 in two populations of Kurds and Lurs with the goal of constructing of a representative Y-STR database including all Iranian ethnic groups.

Blood samples were obtained from 75 Kurds and 50 Lurs unrelated males. Genomic DNA was extracted by a salting-out procedure and loci were amplified in triplex and monoplex PCRs and alleles were identified by comparison with an in-house constructed allelic ladder. Using an implementation of Analysis of Molecular Variance (AMOVA) provided at the YHRD website, Fst value was calculated and the multidimensional scaling analysis (MDS) plot was drawn.

The observed haplotype diversity and discrimination capacity were 0.997 and 90.6% for Kurdish and 0.998 and 94% for Lurs population, respectively.

The most common allele in Kurds/Lurs for respective loci was DYS19, allele 14/14; DYS389I, allele 13/13; DYS390, allele 23/23; DYS391, allele 10/10; DYS392, allele 11/11; DYS393, allele 12/12; DYS385a, allele 13/13; DYS385b, allele 16/16 and DYS389II allele 28/29.

Gene diversity value was calculated from the allelic frequency for each locus. The DYS385b locus proved to be highly polymorphic in both Kurds (0.833) and Lurs (0.824). Also the DYS391 (0.599) and DYS392 (0.264) loci showed the lowest value in Kurds and Lurs, respectively. MDS and Analysis of Molecular Variance (AMOVA) revealed large genetic distances among Kurdish people living in Iran, Turkey and Iraq. Also based on the same analysis a significant genetic distance found between Iranian Lurs and Arabs.

P10.57 ASSOCIATION OF SOD1 GENE ALLELIC VARIANT WITH DIABETIC NEPHROPATHY IN TYPE 1 DIABETES

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Diabetic nephropathy is a major complication of type 1 diabetes whose pathogenesis is insufficiently known, but oxidative stress and genetic susceptibility seem to be involved. The purpose of this study is to assess the possible association of +35A/C (rs2234694) polymorphism in SOD1 gene with advanced stages of diabetic nephropathy in patients with type 1 diabetes in Romania. There have been enrolled 238 unrelated patients, having type 1 diabetes, divided into group A (106 patients) with diabetic nephropathy - macroalbuminuria or ESRD (End Stage Renal Disease) and group B (132 patients) without diabetic nephropathy. The genomic DNA was extracted from the peripheral venous blood and the genotyping of +35A/C (rs2234694) polymorphism has been made using the PCR-RFLP technique. The statistical analysis has been made using De Finetti's program. There has not been a significant deviation from the Hardy-Weinberg equilibrium for none of the groups (p=0.229 respectively p=0.894). The data analysis revealed that the presence of a C allele confers a significant risk (p=0.008) for the advanced diabetes nephropathy (OR=4,940, 95% C.I.=1,341-18,198), and the CA genotype (p=0.015) confers a little lower risk (OR=4,491, 95% C.I.=1,203-16,766). This study shows the association of a mutant C allele of rs2234694 polymorphism in SOD1 gene with the advanced stages of diabetic nephropathy in patients with type 1 diabetes in Romania, suggesting the involvement of the defence against oxidative stress, as an important link in the pathogenesis of diabetic nephropathy.

P10.58 The high SCA1 relative incidence in Poland and the evidence of the founder effect.

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Spinocerebellar ataxias (SCAs) belong to a group of rare hereditary neurodegenerative disorders. In some geographical regions relatively high prevalence of a certain SCA types resulting from a founder effect has been documented. Contrary to the highest worldwide SCA3 incidence, among patients of Polish origin no SCA3 cases were found, although 138 SCA1 and 23 SCA2 and 3 SCA17 pedigrees were identified. The aim of the study was an attempt at finding evidence of possible founder effect in the most frequent types of SCA in Poland.

SCA1 is the commonest genetic type of SCA in Poland with relative frequency of 67% (among all genetically confirmed SCAs) and irregular geographical distribution within the country. Genetic markers: D6S89, D6S109, D6S274, D6S288 linked to the ATXN1 gene were analysed and strong association of D6S89 marker's variant of 197 bp was observed in 81 pedigrees.

In SCA2, which account for 11% of spinocerebellar ataxias in Poland, no association with the four analysed genetic markers spanning the ATXN2 gene was revealed.

P10.59 GALNT2 AND TRIB1 GENES POLYMORPHISMS AND TRIGLYCERIDE LEVELS IN METABOLIC SYNDROME PATIENTS

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Objectives: The rs17321515 was shown to be associated with severe HTG and HLP types 2B, 3, 4 and 5.. The human tribbles-1 (TRIB1) gene is located at chromosome 8q24. In an Asian Malay population, the rs17321515 polymorphism located near 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.

The GALNT2 gene is a member of the GalNAc-transferase enzyme family responsible for the transfer of an N-acetyl galactosamine to the hydroxyl group of a serine or threonine residue in the first step of O-linked oligosaccharide biosynthesis. The rs4846914 is an intronic variant of GALNT2 gene, and its minor G allele showed association with elevated triglyceride levels. A Hungarian case-control study evaluated the association of GALNT2 rs4846914 variant with triglyceride levels, and explored their possible contribution to the development of ischaemic stroke.

Methods and results: A total of 287 metabolic syndrome patients were genotyped by PCR-RFLP. We observed relationships between triglycerides and any genotypes of TRIB1 and GALNT2. Triglyceride levels were higher in minor homozygotes than in two other genotype groups (rs17321515: AA 2.02±0.23; AG 2.01±0.11 GG 2.42±0.25; rs4846914: AA 2.18±0.17; AG 1.99±0.14 GG 2.58±0.33 and p<0.05). The total serum cholesterol and HDL-cholesterol level did not differ between groups of different genotypes.

Conclusions: The analyzed TRIB1(rs17321515) and GALNT2 (rs4846914) SNPs are associated with elevated triglyceride levels in minor allele homozygotes.

P10.60 Genetic structure of rural population Uygur region of Almaty area, of Kasakhstan

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The study of genetic structure of modern populations of the man is one of key problems of genetics. Genetic and demographic information for the uygurs population living in the Almaty area of Kasakhstan Republic is presented. The Uygur region consist of the 14 rural districts. The primary nation amongst the inhabitant in Uygur region were uygurs (56,0%). The marital-migrational structure of Uygur region was studied on the basis of marital recodes. The average value of ethnic marriage assortativeness was found to be 1,68. The respective districts are formed the region's genetic structure of uygur rural population of

Almaty area.

The mean number of children per woman constituted 3,65. Crow index of total selection (Itot) and its components (Im, If) were 0,34, 0,04 and 0,29 respectively. The size of the portion of the population of reproductive age (36,89 % of the total), family size(3,65), and the predominance of the portion of the population (13,39 % of the total) under reproductive allow us to classify this population as growing . The parameters of isolation of Malecot distance and endogamy index in Uygur region of Almaty area of Kasakhstan are counted up. Highest local inbreeding is found in the Avat rural county (0,0089). The index of endogamy in over is 0,41. Recent social and economic changes have led to an increase in general and ethic isolation of uigur rural population of Almaty area of Kasakhstan.

P10.61 Inherited genetic liver disorder impact on VHC infection in Latvia

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Infection rate with viral hepatitis C (VHC) is growing each year worldwide. Hepatitis C virus (HCV) is major cause of chronic liver disease. During antiviral therapy there are frequent adverse effects and negative response to it. Inherited liver diseases as hereditary haemochromatosis, Gilbert syndrome and Wilson disease changes liver cell metabolism and possibly influence HCV infection outcome.

Aims - detect mutations C282Y, H63D and S65C in HFE gene, H1069Q in ATP7B gene mutation, and (TA) repeats in UGT1A1 gene promoter region in HCV patients group and patients group, to evaluate its frequency and impact on clinical outcome.

Material and Methods - DNA samples from 100 VHC patients and 150 healthy individuals.

Results - frequency of H1069Q allele in VHC patients group is 0.055 (in control group 0.06 p=0.0013). Frequency of C282Y allele in patient group 0.067, H 63D - 0.25, S65C - 0.013 - with no significant difference from control population (p>0.05). In gene UGT1A1 promoter region (TA)7 allele was found with frequency 0.402 (in control population 0.37), there were found two alleles (TA)5 in control and patient groups (p>0.05). If patient had at least one of C282Y or H63D mutations during antiviral therapy were elevated ALAT comparing to other patients group p=0.0331.

Conclusions: Allele H1069Q possibly affects host organism acceptability to HCV infection. HFE gene mutations influence adverse effects during antiviral therapy. In Latvia is found (TA)5 allele in gene UGT1A1 promoter region, that has no influence on VHC clinical outcome but is rare in Caucasian population.

P10.62 Genetic structure of Western Caucasus populations on the base of uniparental polymorphisms

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The populations of western part of the Caucasus represent significant historical, ethno-cultural and linguistic traits. We have analyzed 52 markers in coding region of the mtDNA and 48 markers in the non-recombining part of the Y-chromosome in 592 individuals representing five populations from western Caucasus (Abkhazians, Adyghe, Abazins, Georgians, and Circassians). Y-chromosome haplogroups G-M201 and J2 (J-M172) account for more than 50% of all haplogroup diversity in the studied populations. Haplogroup G-M201 in the Western Caucasus populations is represented only by subclade G2a (G-P15) with the insignificantly low exception in the Adyghe population where G1a (G-P20) amounts to less than 1%. In contrast to high frequency of J2 haplogroup J1 exhibit moderate occurrence and vary from 2 to 6 %. Haplogroup R1a (R-SRY10831.2) is also present in all studied populations. It is difficult to say though whether this component is a result of Eastern European influence or it has arrived from other source. While analysis of the coding region with high level of resolution is needed to understand the source of high frequencies of haplogroup H, occurrence of haplogroup U subclades slightly differs in all 5 populations.

P10.63 Analysis of 17 Y-chromosomal STRs loci in the Iranian population

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One of the smallest human chromosomes is the Y chromosome with an average size of 60 Mb. Exchanges is limited to small pseudoautosomal regions of the X-Y pair between X and Y chromosomes in the meiosis. The Y chromosome in most of its length is male-specific and effectively haploid and is transmitted from father to his son unchanged unless a mutational event takes place.

Y chromosome-specific STRs have proved to be an important tool in paternity cases, especially when the alleged father is deceased, as well as forensic and non-forensic fields.

Blood samples were collected from 135 randomly selected, unrelated Iranian males, following procedures that are in accordance with Pro-mega kit and FTA Cards.

All 17 (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a/b, DYS438, DYS439, DYS437, DYS448, DYS458, DYS456, DYS635, and Y-GATA-H4) markers were co-amplified using the AmpFISTR Y-filer™ PCR Amplification kit(Applied Biosystems, USA).The amplified products were separated by capillary electrophoresis on ABI Prism 3130 XL Genetic Analyzer. The sample run data were analyzed by GeneMapper IDX Software V. 1.0. Allele frequencies were estimated. Haplotype diversity was calculated by Nei's formula. To determine of other parameters we are using Arlequin software and also the online AMOVA tool from YHRD.org3.0 for y-STR haplotyping. Allele frequencies in our study are similar to reported allele frequencies in Iranian population on Y-STR haplotype databases. In this study we found some new mutation as off-ladder which most of them are located on DYS458 marker.

P10.64 Hints of positive selection in the promoter of the Coagulation Factor VII gene in populations of Asian descent

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Background.- Immoderate blood clotting constitutes a risk factor for cardiovascular disease in modern industrialised societies, but is believed to have conferred a survival advantage, i.e. faster recovery from bleeding, on our ancestors.

Aim.- Here, we investigate the evolutionary history of Coagulation Factor VII gene (F7) through the analysis of 5 mutations from the F7 promoter associated with the risk for cardiovascular diseases, as well as 6 neutral SNPs from the flanking region in 3 populations of Asian descent (Bolivian Quechuas and Aymaras and Siberian Yakuts).

Methods.- A total of 133 individuals were typed with the iPLEX™ Gold assay on the Sequenom MassARRAY® Platform for the 11 polymorphisms. Population differentiation and selection tests were performed and linkage disequilibrium patterns were investigated.

Results.- No linkage disequilibrium between adjacent mutations -402 and -401 was observed in any of the samples, while the long-range haplotype test detected a potential signal of positive selection for the F7 promoter in the Native Americans, as well as the Yakuts.

Conclusion.- Our data suggest that, in contrast with published data from the Mediterranean region, the F7 promoter may have undergone positive selection in at least some populations of Asian descent.

J10.01 Variability in the 2'-5'-oligoadenylate synthetase gene cluster in human populations from North Eurasia

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2'-5'-oligoadenylate synthetases (2'-5'OAS) are a family of interferon-induced enzymes which play an important role in the antiviral defense

of mammals. In humans there is a cluster of three genes encoding functional synthetases (OAS1, OAS2 and OAS3). Previously we found that five single nucleotide polymorphisms (SNPs) located within OAS2 and OAS3 genes are associated with susceptibility/resistance to severe tick-borne encephalitis virus (TBEV)-induced disease in Russian population. In current study we investigated distribution of the three of that SNPs (OAS3 rs2285932 (C/T, Ile438Ile), OAS3 rs2072136 (G/A, Ser567Ser) and OAS2 rs15895 (G/A, Trp720Ter relative to p71 isoform)) in seven human populations from North Eurasia: Caucasians (Russians and Germans (from Altai region)), Central Asian Mongoloids (Altaians, Khakasses, Tuvinians and Shorians) and Arctic Mongoloids (Chukchi). Highly significant differences between populations in genotype, allele and haplotype frequencies for these SNPs were detected. Moreover, we found that these frequencies correlate not only with the ethnicity of the populations but also with their differential exposure to TBEV during their evolution. For example, G/G genotype for OAS3 gene rs2072136 SNP (that according to our previously obtained data "predispose" to severe TBEV-induced disease) was found with the lowest frequencies in Altaians, Khakasses, Tuvinians and Shorians who highly contacted with TBEV during their ethnogenesis. Thus, data obtained allow to suppose that TBEV might act as a selection factor in these populations.

J10.02 Analysis of genetic polymorphism of TCF7L2 gene of elderly people, residents of besieged Leningrad.

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TCF7L2 gene encodes a high mobility group (HMG) box-containing transcription factor that plays a key role in the Wnt signaling pathway. This protein implicates in blood glucose homeostasis. Genetic variants of this gene are associated with increased risk of type 2 diabetes, insulin resistance. We suggest that ability of this gene to play a part in storage of energy could help to survive of besieged Leningrad residents. Using RFLP method polymorphism of TCF7L2 (rs7903146, IVS3C>T) gene of 97 elderly people was analyzed. This polymorphism was investigated in 2 groups: 26 elderly people were survived in condition of besieged Leningrad (Group1) and 71 elderly people (group2) from North-West region of Russia. Distribution of genotypes of TCF7L2 gene was significantly different between groups ($p=0.046$, $df=2$). Increasing of T allele in group1 compared with group2 (26.9% and 12%, accordantly, $\chi^2=6.34$, $p=0.01$) was founded.

It could be speculated that person with certain T allele of TCF7L2 genes has some metabolic advantages for longer survival in condition of besieged Leningrad. Further it is necessary to perform studies of more numerous groups of different age to estimate the role of age-regulating genes in condition of besieged Leningrad.

J10.03 Analysis of TGF β 1 gene polymorphism in Balkan Endemic Nephropathy

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Balkan endemic nephropathy (BEN) is a multifactorial disorder with still unexplained hereditary component. Similarity of BEN and cyclosporine nephropathy suggests possible common ethiopathogenetic mechanisms. Considering the role of transforming growth factor β 1 (TGF β 1) in kidney disease, and the link to renin-angiotensin-aldosterone system (RAAS), we performed analysis of G915C polymorphism in TGF β 1 in BEN patients. It is known that the less common C allele of the TGF β 1 polymorphism is associated with a lower TGF β 1 production capacity.

The study was carried out in a group of 50 patients with BEN diagnosis according to criteria of Danilovic, derived from endemic region in Kolubara district, Serbia. Two control groups consisted of 50 healthy persons (C) and of 45 patients with other nephropathies (NBEN), both matched by age and gender. DNA for gene analysis was extracted from peripheral blood leukocytes. For detection of TGF β 1 G915C gene

polymorphism PCR/RFLPS method was used. *TGFβ1* serum level and urine excretion in some patients were also measured by ELISA. We found that frequency of *TGFβ1* CC genotype was 33.33%, 14.29% and 33.33% in BEN, C and NBEN group, respectively. Our results showed significantly higher frequency of *TGFβ1* CC genotype in both BEN and NBEN group than in C group. There was correlation between *TGFβ1* genotypes and serum levels of that growth factor. The obtained results indicate the significant role of immune mechanisms in emergency of kidney diseases such as BEN and other nephropathies, and could help the further investigation of genetic factors that contribute to BEN pathogenesis.

J10.04 The combination of rare alleles of some gene polymorphism in children and adolescence with risk factors of Cardio Vascular Disease (CVD)

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Objectives. To study rare alleles of gene polymorphisms intron4 eNOS, I/DACE, W64RADRB3, Q/E2ADRB2, G/R16ADRB2, G-75AApoA1, C+83TApA1, SstApC3, S19WApoA5, -1131T<CApoA5 distribution in children and adolescents with risk factors of CVD.

Study population. We included 100 children and adolescence, whose parents suffered SVBD and having risk factors of CVD, age of 5-17 years (68 males, 32 females).

Control group included 145 children and adolescents (73 males, 72 females) of the same age.

Methods. The analysis of intron4 eNOS, I/DACE gene polymorphisms was performed by PCR. G-75AApoA1, C+83T ApoA1, SstApC3, S19WApoA5, -1131T<CApoA5, W64RADRB3, Q/E27ADRB2, G/R16ADRB2 gene polymorphisms were determined by PCR-LRFP method.

Results. Significant differences were detected between the groups in frequency of Q/E27ADRB2 gene polymorphism Q27-allele 0,57 vs 0,44 and E27-allele 0,43 vs 0,56 (n=100 vs n=145 respectively; p=0,02)

Results of combination of rare alleles investigated gene polymorphisms are: one rare allele - 2% vs 17,9%, 2 rare alleles- 17% vs 22,1%, 3 rare alleles - 29% vs 25,5%, 4 rare alleles 26% vs 15,2%, 5 rare alleles - 13% vs 6,2%, 6 rare alleles 12% vs 4,1%, 7 rare alleles 1% vs 0%, 8 rare allele 0% vs 0,7% (n=100 vs n=145 resp.). In control group 9% of children and adolescence haven't rare alleles of investigated gene polymorphism.

Conclusions: In group of children and adolescence, whose parents suffered SVBD and having risk factors of CVD, frequency of E27 allele gene polymorphism Q/E27ADRB2 was higher. Combination of 3-6 rare alleles investigated polymorphisms can be considered as additional risk of CVD.

J10.05 The impact of genetic variability and smoking habits on the prevalence of periodontitis among adults

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Aim : Elucidate the effect of genetic variance of inflammatory mediators expression ,the influence of microbial expression, and smoking as a risk factors for periodontitis

Material &Methods: Sample of this study composed of 50 smokers & 50 non smoker volunteers (unrelated and of the same ethnic population) with 40-60 years old .Their periodontal status was estimated through periodontal examination (full mouth clinical attachment loss measurement ,probing depths ,plaque index scores, and bleeding on probing). Isolation and detection of certain oral pathogens; A.actinomycetemcomitans , Porphyromonas gingivalis ,and Prevotella intermedia was performed . Genotype for bi-allelic IL-1A+4845, IL-1B+3954 gene polymorphisms using mouth wash was detected by PCR based methods.

Results: There were a significant difference only between the two groups (smokers &non-smokers) as regards to colonization of

A.actinomycetemcomitans & not among Porphyromonas gingivalis & Prevotella spp. There were no significant difference between the overall frequencies of carrying allele 2 of IL-1 A, IL-1B among smoker and non-smokers. The percentage of non smokers having healthy periodontal status was much higher than smokers. On the other hand, smokers recorded much higher percentage for mild, moderate and severe periodontitis. The difference was statistically significant concerning the percentage of those with severe periodontitis.

Conclusion: Environmental factors play either a direct (i.e., causative factor) or indirect (modifying factor) role as a risk factor for periodontitis. The association between genetic polymorphism of allele 2 of IL-1A, IL-1B expression & smoking habits caused a synergistic effect for progression of periodontitis. Smoking initiated A.actinomycetemcomitans growth.

J10.06 ANALYSIS OF POLYMORPHISM AT THREE NUCLEAR GENOME DNA LOCI IN UIGURS

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The polymorphic nuclear locus in human genome are the objective genetic markers for the analysis of hereditary polymorphism of the modern human populations and evolution.

A total of 131 unrelated healthy Uigur people of Kazakhstan were studied. In lust studies the genotypes and allelic frequencies of ACE, PV92, TRA25, APOA1, YaNBC148, YaNBC27, CCR5 genes were revealed. The distribution of the empirical genotypes and allelic frequencies and the indexes of heterozygosity of all genes were completely conformed to theoretical of Hardy-Weinberg equilibrium (p>0,05), except for PV92 gene ($\chi^2=10,6$; p<0,001), for TPA25 gene ($\chi^2=5,1$; p<0,001), for YaNBC148 gene ($\chi^2=5,1$; p<0,001).

The genotypes and allelic frequencies of TNFa gene were: GG - 36.6%; AA - 1.5%; GA - 61.8%; G - 67.6%; A - 32.4%. The frequencies of TP53 gene's genotypes and alleles were: PP - 38,2%, AA - 15,3%, AP - 46,6%, P - 61,5%, A - 38,5%; and of ITGB3 gene's were: LL - 19,1%, PP - 10,7%, LP - 70,2%, L - 54,2%, P - 45,8%.

The frequencies of TP53 gene's genotypes were corresponded to Hardy-Weinberg equilibrium completely (p>0,05); the genotypes of TNFa and TGB3 gene's frequencies weren't corresponded to Hardy-Weinberg equilibrium ($\chi_1^2=20,3$; p<0,0001 for TNFa and $\chi_2^2=20,9$; p<0,0001 for ITGB3 gene).

Thus, the results of our research are evidence of the probable compound ethnic history of Uigur people.

J10.07 A short tandem repeat polymorphism in the inducible nitric oxide synthase gene in Buryat and Russian populations.

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Nitric oxide (NO), an important molecule in various biologic processes that include airway inflammation is synthesized from L-arginine by isoforms of NO synthase (NOS). Overproduction of nitric oxide (NO) by inducible NO synthase (iNOS) has been implicated in the pathogenesis of several diseases including airway inflammation of asthma. A highly polymorphic pentanucleotide (CCTTT)n repeat located in the iNOS gene promoter region has been shown to be functionally important in the regulation of iNOS transcription.

Genotype and allele frequency of (CCTTT)n repeat in the promoter region of the iNOS gene, were analyzed in 90 healthy Buryat individuals and in 107 Russian individuals. The Buryats are the ethnic minority group of Siberia, which belongs to Mongolic group and the Russians belong to East Slavic peoples. We have identified significant difference in allele frequencies of repeats between these ethnic groups. In Russians 7 alleles with 9-15 repeats were identified, whereas 10 alleles with 10-19 repeats were revealed in Buryats. The most common allele in Russians was 12-repeats (26,6%), in Buryats was 14-repeats (13,9%). Alleles of 17, 18 and 19 repeats were detected only in Buryat samples. According to literature data 17-repeats, 18-repeats and 19-repeats had been reported previously in Chinese population and had not been reported in Caucasians. The panel and the frequencies of studied iNOS alleles in Russian sample correspond to those ones in Caucasians.

J10.08 Distribution of the ABO Blood Groups and RH in KAZAKH.

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The gene pool of any ethnic group it is a result of centuries-old genetic significant demographic transformations. Therefore it is represented interesting to carry out research of genetic structure of population of Zhambyl raion (district) of Almaty region of Kazakhstan on blood type ABO and Rhesus factor markers. Research materials: archival records of the Kazakh patients of the hospital of Zhambyl raion of Almaty region (N =3112).

The parity allele's ABO is presented by the formula: ABO*O > ABO*A > ABO*B > ABO *AB. In the Kazakh population the phenotypes frequency were as follows: ABO*O = 36.18%, ABO*A = 30,17%, ABO*B = 25.42%, ABO*AB = 8.23% and RH*d = 1.06%. In the rural population the alleles frequencies were as follows ABO*O = 0.599; ABO*A = 0.215; ABO*B = 0.185 and RH*d = 0.103. In Kazakh population the heterozygosity (h_e) indices in respect to the ABO and RH alleles were 0.560 and 0.185, respectively.

The rural population of Zhambyl raion the heterozygosity indices in respect to the ABO and RH alleles were lower than the some rural populations of Almaty region. Nevertheless, distribution of the phenotypes frequency of the ABO and RH keeps within the variations peculiar to the population Central and the Central Asia, in some cases coincides with those variations on the people of the Eastern Europe and Forward Asia, but the heterozygosity were differs for Russian of Moscow and Ukrainians of Donetsk region.

J10.09 The I/D polymorphism of ACE gene may be involved in the left ventricular (LV) remodeling process

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Background: LV remodeling is the manifestation of a target organ damage from arterial hypertension (AH). The role of renin-angiotensin system (RAS) genes in the remodeling process has been studied in adults.

The aim of this study was to evaluate the RAS gene polymorphism in LV remodeling process in children with AH.

Methods: 82 children aged 5-17 with systolic AH were studied. LV mass was calculated by Devereux formula. LV mass index was calculated by dividing LV mass by height^{2.7}. The sex-specific 95th percentile for LV mass index in normal children and adolescents was used as a cutpoint (Daniels et al., 1995). Relative wall thickness was considered abnormal when it was 0.41. The I/D polymorphism of ACE gene, M235T polymorphism of AGT gene, and A1166C polymorphism of AGTR1 gene were detected with PCR-RFLP.

Results: Concentric LV remodeling was found in 7 (9%) patients, concentric hypertrophy - in 6 (7%), eccentric hypertrophy - in 10 (12%), and normal LV geometry - in 59 (72%) patients. The genotype DD frequency of I/D polymorphism of ACE gene was significantly higher in children with concentric LV remodeling compared to children with normal LV geometry (57% vs 22% p < 0.05). No significant difference was found between genotypes and allele frequency of both AGT and AGTR1 gene polymorphism in patient according to their LV geometry. Conclusion: LV remodeling was found in 23 (28%) of hypertensive children. We suggested that the I/D polymorphism of ACE gene may be involved in the LV remodeling process.

J10.10 THE ANALYSIS OF THE GENE POOL OF KAZAKH

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The goal of the present research was: to study the polymorphism of mitochondrial DNA and DNA of the nuclear genes in Kazakh population.

We have analyzed the 129 polymorphic positions, 44 different haplogroups (hg) of mtDNA of 304 Kazakh mtDNA samples. The variability index of haplotypes was 0,99 and the 80,7% unicum haplotypes were

determined in Kazakhs.

We have found that more than 55% of mtDNA lineages belong to East-Eurasian specific hgs (D, C, G, A, M, F). The supercluster D was found with high frequency (18,1%). The frequencies of the hgs were: C - 9,5%, G - 6,6%, A -3,0%; M -2,6%; F -3,6% were determined in Kazakhs.

The Western-Eurasian specific hgs (H, T, J, K, U2, U5, HV) were observed in Kazakhs with frequency 41%. Hg H was found with frequency 14,1% . The frequencies of the hgs T (3,9%), J (3,6%), U5 (3,0%), K (2,6%), W (1,6%) V (0,7%) and I (0,3%) were found in Kazakhs populations.

We studied 5 polymorphic locus of Alu-insertion : ACE, TPA25, PV92, APOA1, NBC27 in population of Kazakhs (n=224). We observed frequencies of insertion for ACE, APOA1, PV92, TPA25, NBC27, and their frequencies were respectively 51,3 %, 85% ,52,7%,48,4 %,28,3 % in Kazakhs.

The obtained data may prove useful in studies of ethnogenetic history of Kazakhs. The obtained data allowed us to construct a phylogenetic tree for Kazakhs on female lineage and to detect position of the studied population between ethnic groups in Europe and Asia.

J10.11 MtDNA and Y-chromosomal variation in populations of Sakha (Yakutia)

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We have characterized, at high phylogenetic resolution, mtDNA variation (n=694) and Y chromosome haplotypes diversity (n=318) in populations of Sakha (Yakutia) -the Autonomous Republic situated in northeastern part of the Russian Federation that comprises 1/5 part of Russia's total land area. The results were analyzed in a broader context of the Eurasian mtDNA and Y-chromosomal variability. Extended analysis confirms that Yakutia was colonized from the regions west and eastward of Lake Baikal with minor gene flow from Lower Amur/Southern Okhotsk region and/or Kamchatka. The genetic portraits of studied ethnic groups (Sakha or Yakuts, Evenks, Evens, Yukaghirs, Dolgans) were obtained and scenarios of ethnogenesis suggested by historians and archaeologists were compared with genetic reconstructions. We considered our results in connection with some epidemiologic and molecular genetic researches of hereditary diseases characterized by a high prevalence in the region such as spinocerebellar ataxia type 1, myotonic dystrophy, 3-M syndrome.

J10.12 The C677T and A1298C Mutations in the Methylenetetrahydrofolate Reductase Gene among the Kazakh Population

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The presence of the C677T and A1298C mutations in the methylenetetrahydrofolate reductase (MTHFR) gene has been regarded as a genetic risk factor for neural tube defects, some complication during the pregnancy and coronary artery disease.

The prevalence of their mutations has been reported from various populations. We have investigated the frequencies of MTHFR mutations in 303 unrelated Kazakhs and compared our results with the findings of other researchers. The frequencies of C677T mutation were: CC - 59,1%, CT - 39,4% and TT - 1,6%. Kazakh population, as well as Canadian Inuit, South African and sub-Saharan African, show the lowest TT genotype in this study. According to a previous report of other authors the Mexican population has a highest frequency of TT genotype (34,8%). The frequencies of A1298C mutation of Kazakh were: AA -55,2%, AC - 38,4% and CC - 6,4%. The prevalence of CC homozygosity wasn't rare amongst Kazakhs and hasn't significant differences from other world populations.

In addition we revealed the frequencies of MTHFR mutations in 84 women with fetuses with various neural tube defects. The frequencies of C677T mutation were: CC -48,8%, CT -42,9% and TT - 8,3%. The frequencies of A1298C mutation Kazakh were: AA -44,0%, AC - 41,7% and CC -14,3%. Thus, homozygosity for C677T and A1298C mutations in the MTHFR gene may be a possible genetic risk factor for neural tube defects in the Kazakh population.

J10.13 Research of hereditary ophthalmopathology of the Rostov Region, Russia.

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A genetically epidemiological examining of the population of Rostov Region (twelve districts) was implemented. Total size of investigated populations was 454524 persons (90% Russians). The research was conducted under the original protocol, providing for detection of more than 2500 various hereditary diseases and syndromes, including all hereditary ophthalmopathology. Hereditary eye pathology is a large heterogeneous group of genetic diseases with different prevalence rate in various populations. Hereditary diseases eye were diagnosed as isolated ones, and they were also a part of hereditary diseases and syndromes. Prevalence rate of isolated hereditary eye diseases was 4,6 (with variation for districts from 0,5 to 9,6 per 10000 of population). The most frequent hereditary pathology eye was retina degeneration the prevalence rate of which made up 1,3 (with variation for districts from 0,2 to 4,5 per 10000). Various forms of congenital cataracts (cataract lamellar, cataract-microcornea syndrome, microphthalmia with cataract) were ranked second as per their prevalence. Total prevalence rate there of was 1,3 (from 0,2 to 2,8 per 10000). Prevalence rate of congenital monogenic malformations of eye was as follows: coloboma of iris 0,2 (with variation for districts from 0,2 to 1,3 per 10000), aniridia 0,1 (from 0,2 to 1,7 per 10000), ptosis hereditary congenital 0,5 (from 0,2 to 2,1 per 10000). Ehlers-Danlos, Marfan, Usher, Sturge-Weber syndromes, osteogenesis imperfecta were the most frequent mono-genic syndromes followed by eye pathology. Prevalence of hereditary diseases of retina and congenital cataracts in the Rostov region turned out higher than in other previously examined populations of Russia.

J10.14 Analysis of uncoupling protein 2 and 3 genes polymorphisms in elderly people, residents of besieged Leningrad.

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Our goal was to investigate whether polymorphisms in UCP2 and UCP3 genes influence life expectancy. UCP2 and UCP3 belong to mitochondrial carrier protein family of genes which provides opportunity to study thermogenesis in humans. These genes supervise heat exchange which influences preservation of energy necessary not only for living, but also survival in extremely situations.

We have analyzed UCP2 Ala55Val and UCP3 C-55T polymorphisms by RLFP method of 133 elderly people (group 1) and also of elderly 46 residents of besieged Leningrad (group 2) from North-West Region of Russia. Distribution of genotypes of UCP2 gene was significantly different between groups ($p=0.032$, $df=2$). Increasing of Ala allele in group 1 compared with group 2 (23.3% and 6.5%, accordingly, $\chi^2=6.26$, $p=0.012$) was founded. No significant difference between genotypes of the C-55T polymorphisms in the UCP3 gene was found. However, we are registered increasing frequency of T allele in group 1 compared with group 2 (33.8% and 26.1%, accordingly, $p=0.098$).

Consequently we suggest that UCP2 Ala55Val polymorphisms are significant for survival. It also seems interesting that Val allele which is associated with reduced thermogenesis is prevalence in elderly residents of besieged Leningrad. On the basis of these results we suppose that those who have Ala allele, have less charge of heat.

P11 Genomics, Genomic technology including bioinformatics methods, gene structure and gene product function and Epigenetics

P11.001 Genetic analysis of ACE polymorphisms in children with secondary high blood pressure

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Several genetic investigations have been attempted to elucidate the association of angiotensin-converting enzyme (ACE) gene polymorphism and arterial hypertension. The essential role of the renin-angiotensin system (RAS) in controlling blood pressure has been well established. Genes encoding components of the RAS have been proposed as candidate genes that determine genetic predisposition to hypertension. The purpose of this study was to analyze angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphisms in romanian hypertensive children in Mures region. Twenty hypertensive children (1-17 years old, blood pressure ≥ 95 th percentile for age, sex and height) and a control group of thirty normotensive children were included in the study. We analyzed the ACE gene I/D polymorphism by using a polymerase chain reaction assay, and agarose gel electrophoresis system. The results of the study showed that the frequency of DD, ID and II genotypes were 21,43%, 42,86% and 35,71 % in hypertensive group respectively 46,67%, 43,33% and 10 % in control group with significantly higher frequency of II genotype in patients as compared to the control group. The frequency of ACE II genotype in patients with secondary hypertension (35,71%) was significantly higher than in controls (6,67%) in the men. This result suggested that ACE II genotype may be associated with secondary hypertension in the men, not in the women, and showed the possibility of ACE II genotype as a potent risk factor for secondary hypertension for the men not for the women. Acknowledgments: This study was supported by a CNCSIS Grant - 137/2008 for Young PhD, Romania.

P11.002 Study of the ACVR1 gene expression and regulation.

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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 data available in GenBank, ACVR1 has two main transcripts differing for their 5'UTR end. Our bioinformatic analysis of the genomic region containing the gene reveals the presence of several ESTs, predicting the existence of multiple transcripts in which different 5'UTR exons are combined to a common coding sequence. The 3'UTR region is common to all transcripts and contains AU-rich elements and putative, well-conserved binding sites for miRNAs.

Following the above prediction, we found transcripts with different exon composition at the 5'UTR and show their expression profile in different tissues. These data suggest complex regulation, with different transcription start sites and promoter regions and possible elements controlling transcript stability or translation.

Functional analysis of the 3'UTR region by Luciferase reporter assays revealed a negative role in gene expression. ACVR1 transcript, assessed by quantitative PCR after treatment with inhibitors of transcription, appeared unstable. We show experiments that demonstrate negative regulation by miRNAs: transfection of Pre-miR miRNA Precursor Molecules in presence or absence of specific Anti-miR miRNA Inhibitors showed an effect of mir-365, -148b, -384, -381 on ACVR1 transcript, quantified by quantitative PCR.

Our results highlight the complexity of transcriptional and post transcriptional regulation of ACVR1 gene expression.

P11.003 Correlation Between Transposon-Derived Repeats and Markers of Germline Methylation in the Human Genome

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A potential relationship between transposon-derived repeats (TDR) and human germline methylation is of biological importance since it could affect the expression and function of nearby genes. Furthermore, DNA methylation has been proposed to serve as a defense mechanism against genome stability threats posed by TDR. Using a

recently published marker of germline methylation, density of HapMap methyl-associated SNPs (mSNPs), we studied the correlation of the marker and regional proportion of TDR. After correcting for confounders, we found a strong negative correlation between proportion of Alu repeats and mSNP density for 125-1000 kb windows. However the correlation between the proportion of L1 repeats and mSNP density was negligible in 125-500 kb windows, but strong and negative for the largest 1000 kb windows. Using bisulfite sequencing methylation data from the Human Epigenome Project on sperm, the final product of the male germline, we found a lower proportion of Alu repeats adjacent (3-15 kb) to hypomethylated amplicons. In contrast, we found a higher proportion of L1 repeats adjacent (3-5 kb) to hypermethylated amplicons.

Our results indicate that DNA methylation is unlikely to be a global defense system against genome stability threats by TDR. Although a cause-and effect relationship should not be determined using correlation data, we offer the most parsimonious explanation of our results. It suggests the germline insertion of the two major human repeat families (L1 and Alu) into hypomethylated regions. This is followed by post-insertional methylation of sequences adjacent to L1 repeats or post-insertional selection against L1 repeats in hypomethylated regions.

P11.004 Iron/Copper metabolism in Mild Cognitive Impairment (MCI) and Alzheimer's Disease (AD)

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Alzheimer's disease is the most prevalent neurodegenerative disease. Patients with MCI are at higher risk for progressing to dementia of the Alzheimer type, and therefore represent an important population to understand AD and early neurodegeneration mechanisms.

In this study, we further investigated the implication of the redox-active biometals, copper (Cu) and iron (Fe) imbalance in the oxidative injury hypothesis of AD pathogenesis by: (I) comparing serum biochemical markers of Fe/Cu metabolism in a sample of 73 AD patients, 24 MCI patients and 60 controls; (II) testing, in the same sample, a set of Fe/Cu metabolism-related genes and *APOE* for association with MCI/AD. Significant differences were found between AD patients and controls for serum Fe concentration ($p=0.001$) and transferrin saturation ($p=0.007$). A significant association with AD was found for *TF* - transferrin gene ($p=0.0082$) and for the first time for *SLC40A1* - ferroporin (Fpn) gene ($p=0.0355$). *APOEε4* was also significantly associated with AD ($p=0.0004$), in agreement with previous studies. Statistical analysis is underway for the MCI population.

We hypothesize that the lower serum Fe concentration observed in AD patients can be due to impaired Fe excretion from cells, since Fpn is the only known Fe exporter in mammalian cells. The intracellular accumulation of Fe, particularly in the brain, where Fpn is also expressed, would lead to a rise in oxidative damage, contributing to the AD physiopathology. The advent of using a pre-AD state such as MCI may further contribute to elucidate the involvement of Fe metabolism in AD.

P11.005 An automated analysis protocol for aneuploidy detection of QF-PCR data generated on capillary electrophoresis instruments

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Molecular methods for aneuploidy analysis have become important tools for the study of chromosome abnormality in conditions such as Down syndrome, Edwards syndrome and Patau syndrome.

Researchers who routinely perform this type of research have a need for automation of data analysis and concise report generation. An innovative strategy for the detection of chromosomal aneuploidy at the molecular level is analysis of short tandem repeat markers using quantitative fluorescence PCR. Such techniques will help diminish workflow bottlenecks introduced by more time consuming, conventional research methods. This poster will present an automated procedure for analysis and report generation for aneuploidy detection of chromosomes 13, 18, 21, and of sex chromosomes X and Y, in sample

data generated on capillary electrophoresis instruments using fragment analysis software tools. We will demonstrate how key features in the software, such as sample quality values, allele binning and report analysis, enable this workflow to be a significant improvement over traditional detection methods.

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

P11.006 Two new cases of 6q16 microdeletion: clinical findings and cognitive profile associated

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Introduction: most patients with interstitial deletion at 6q16 showed hypotonia and developmental delay, some of them have also a Prader-Willi-like phenotype with obesity. Here we present detailed clinical and cognitive findings in two new patients with 6q16 microdeletion.

Material and methods: whole genome array-CGH was performed on Agilent oligo-chip 44K. Microsatellite markers were used to confirm the array-CGH findings. A number of neuropsychological tests were performed to establish the cognitive-behavioural profile of the patients.

Results: in a series of 200 patients with mental retardation associated with congenital anomalies, we found two patients with an interstitial deletion in 6q16. Both deletions occurred de novo in the paternal chromosome, and are very similar in size (9-10 Mb) and position (from position 93,932,682 to 104,244,422). Detailed cognitive-behavioural profile showed interesting similarities with a generalized cognitive deficit in all the tested areas specially in language and fine motor skills tasks. In behaviour highlights: cognitive slowing and attention deficits.

Discussion: The clinical characteristics of these two new cases of 6q16 deletion are compared with those previously reported cases with similar deletions. We also propose that this anomaly could be associated with a characteristic cognitive-behavioural profile.

P11.007 Fine-mapping analysis of the 5q35 region through a 60K customized CGH-array in patients with Sotos syndrome.

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Sotos syndrome is an overgrowth disorder characterized by excessive growth during childhood, macrocephaly, distinctive facial gestalt, learning difficulty and other variable minor features. The diagnosis is suspected after birth because of excessive height and head circumference, advanced bone age, hypotonia and feeding difficulties. In Europeans, mutations (85%) and deletions (15%) of the *NSD1* gene located at chromosome 5q35 (coding for a histone methyltransferase implicated in transcriptional regulation) are responsible for the majority of cases.

Sixteen out of 101 patients with Sotos syndrome have deletions encompassing the *NSD1* gene. All patients were initially diagnosed with a combination of MLPA and/or microsatellite analysis. A customized 8x60K oligonucleotide CGH array, spanning the 5Mb surrounding the *NSD1* locus, was designed to evaluate the critical Sotos syndrome region.

Four patients had very small deletions (3.8-12.2 Kb) affecting some *NSD1* exons (1,2, 5, 9-12) and the remaining individuals showed larger deletions (0.8-2 Mb) including the entire *NSD1* and some neighbour genes. In addition to the classical deletion, one patient also had distal 5q35 duplication encompassing *ADAMTS2* and *TRIM41*, suggesting a different mechanism of rearrangement of the distal chromosome 5. Oligonucleotide CGH-array is a useful tool for fine-mapping analysis of the 5q35 region in patients with Sotos syndrome and microdeletions. Partial, small deletions of the *NSD1* gene and large deletions affecting other genes can be correctly diagnosed with this technology. Further comparative studies of patients with well characterized genotype, phe-

notype and microdeletion sizes and breakpoints will help to determine possible influence of clinical outcomes.

P11.008 The study of DNA methylation patterns in the forkhead transcription factor (Foxp3) gene in asthmatics and controls

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Background: DNA methylation is the most frequent and stable form of epigenetic modifications. Epidemiological evidence increasingly suggests that environmental exposures early in development have a role in susceptibility to disease in later life.

DNA methylation of genes critical to T-helper cell differentiation may induce polarization toward or away from an allergic phenotype like asthma. One of these genes is Foxp3, which is specifically expressed in T regulatory cells (Tregs) and plays a central role in Treg development and function. Methylation leads to less Foxp3 expression and reduced Tregs function. Asthma is probably characterized by a deficiency in Tregs allowing TH2 cells to expand.

Aim: To investigate the impact of environmental exposures on the methylation status of asthma candidate genes and on the development and severity of asthma phenotypes later in life.

Methods: The detection of DNA methylation is based on the ability to distinguish cytosine from 5-methylcytosine in the DNA sequence and is performed through chemical modification of DNA by bisulfite treatment, followed by PCR, base-specific cleavage and analyses of the cleaved fragments by MALDI-TOF mass spectrometry (Sequenom-EpiTYPER Software).

Results: The methylation patterns of the Foxp3 gene will be investigated in a subset of the KORA survey (F4; 100 asthmatics and 100 controls). First results point to no methylation differences in human blood between asthma cases and controls.

P11.009 Unblocking the flow of genetic variation data - a variant browser case study

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As part of the EU framework 7 project Gen2Phen we are working to provide effective mechanisms to allow genetic variation data to flow between systems. There is a large quantity of such data available, but it is stored, accessed and presented in heterogeneous ways. In most cases it is useful for human consumption, but difficult to access for machines. Moreover, there are social and legal issues involved which complicate the sharing of this data. With the increasing necessity of automated analyses, and the resulting need to integrate data into a range of bioinformatics applications, it is important to address this data flow problem. Here, we discuss some of the issues involved and the solutions that the Gen2Phen partners are developing. These include web service interfaces for machine-to-machine communication, a model and format for exchange of variant data and a reference sequence standard which provides a means to record variation in an unambiguous and immutable fashion. We demonstrate the use of these technologies through their integration into a gene browser application (the NGRL Universal Browser) which provides a unified view of variation data from multiple sources. We assess what improvements in data flow have been made by comparing with our own experiences of performing the same data integration tasks before the contributions of Gen2Phen and after. We also identify improvements that could further streamline the flow of data in the future.

P11.010 *In silico* analyses of promoter regulatory targets in the iron metabolism pathway

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Aim: Investigate the promoter region of genes of the iron metabolism pathway by employing comprehensive bioinformatic analyses, in order to elucidate specific mechanisms of gene regulation.

Introduction: The human genome is a system regulated at many different levels. Transcriptional regulation is the first, and arguably the most important, step in the process of gene expression. This process is gov-

erned by the presence of specific *cis*-regulatory regions (*cis*-motifs) residing within the promoter region of genes and the functional interactions between the products of specific regulatory genes (transcription factors-TFs) and these *cis*-motifs. Bioinformatic tools can be utilised to formulate putative predictions on how specific *cis*-motifs may influence the expression patterns of specific genes or groups of genes (e.g. iron metabolism pathway).

Method:

- Retrieve 2kb 5-prime UTR (promoter) sequences from the human Ensembl database.
- Conserved Nucleotide Sequence (CNS) analysis of promoter regions using specific software tools (e.g. VISTA).
- Computational analysis and *in silico* design of promoter models using probabilistic detection methods such as expectation maximization (MEME).
- Refined analysis and identification of detected motifs analyzed using TRANSFAC and JASPAR.

Results: Specific and/or combinations of *cis*-motifs were discovered within a CNS identified in the promoter of seven of the tested iron genes. Within these motifs, relevant tissue- and pathway-specific TF-binding sites were predicted to occur.

Discussion: Accurate analyses of *cis*-motif architecture combined with integrative *in silico* modelling (once validated by experimental analysis) could offer insights into complex mechanisms governing transcriptional regulation, serving as a refined approach for prediction and study of regulatory targets.

P11.011 Analysis of *in-silico* tools for the prediction of pathogenesis in missense variants

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Missense variants occurring in disease related genes can often cause pathogenesis by mutating the protein product of the gene. However, missense variants can also occur with non-pathogenic effects, coding for amino acid changes which do not significantly affect the protein product. It is often very difficult to determine whether an unknown variant is pathogenic or not. *In silico* tools can predict the pathogenicity of a variant based on the change in amino acid. These tools use features such as the evolutionary conservation of the amino acid position or the physical characteristics of the amino acids such as size, hydrophobicity and polarity.

Although a large number of tools exist which aim to predict the pathogenesis of a missense mutation, only a small number of these tools are regularly used by laboratory scientists. This study aims to benchmark these, and other, prediction tools and to provide guidance about which are the most reliable.

Ten prediction algorithms have been chosen for the analysis. The ability of these tools to accurately predict both pathogenic and non-pathogenic missense mutations was assessed. The performance was assessed using a dataset consisting of 1656 pathogenic variants and 570 non-pathogenic variants, retrieved from locus specific databases (LSDBs) for 26 different genes. The results of the analysis show that while some of the tools currently used by laboratories are high performing, other less well known tools may be a useful addition to the bioinformatics analysis of unknown missense variants.

P11.012 Haplotype Specific Amplification in High Throughput Tumor Sequence Data

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During tumor progression, culprit genes and variants confer selective advantage to progenitor cancer cells via allowing them to bypass normal growth control mechanisms. Regions of somatic amplification represent one such variant type and are a hallmark of tumor genomics. When integrated with single nucleotide polymorphism (SNP) information at the germline level - the second variant type - causal oncogenes may be localized within such regions. This integration was explored previously on SNP arrays using allele specific copy number calls at each marker. However, the relative sparseness of SNP arrays and the fidelity of these calls leave room for improvement. The advent of high

throughput sequencing technologies provides an exciting opportunity to examine massive amounts of genomic data at the nucleotide resolution. We propose a novel Hidden Markov Model-based method that analyzes tumor sequence data to infer amplified alleles and haplotypes in pre-determined somatically amplified regions. Our method is designed to handle biases in read data as well as handle rare variants. Furthermore, it utilizes existing information from public repositories, such as the 1000 Genomes Project, when inferring haplotypes. We thus believe our method will help further the integration of variant types in sequence data and aid the cancer community in identifying causal variants.

P11.013 Cellular expression of human plakophilin-2 mutations suggests the involvement of proteolytic degradation in the disease process of arrhythmogenic right ventricular cardiomyopathy

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetically determined heart muscle disorder characterized by progressive loss of cardiomyocytes and fibro-fatty replacement. The armadillo protein plakophilin-2 (PKP2) has been identified as the most prevalent disease gene. To get further insights in the underlying genetic mechanisms of these mutations and to investigate their influence on cellular junctions integrity, protein stability and cell signalling we over-expressed different human missense (C796R, S615F, K654Q) and frame-shift (C693fsX741) mutations in epithelial cell lines (A431, HeLa-M2).

In contrast to the wild-type protein and two unclassified variants (V587I, I531S), which showed the expected localization at the junctional plaque, the mutant proteins demonstrated a cytoplasmatic expression pattern with accumulation around the nucleus. Western blot analysis demonstrated significantly lower levels of the mutant PKP2 proteins. Additionally we investigated direct interactions of mutant PKP2 with desmoplakin (DSP) by co-expressing both proteins. In contrast to the proper membrane associated localization of PKP2 and DSP after cotransfection of both wild-type proteins - mutant PKP2 proteins were not able to interact with DSP to enable the assembly at the junctional plaque. This indicates the requirement of functional PKP2 for DSP integration in to the desmosome.

To prevent the potential degradation of mutated PKP2 proteins we used different inhibitors of the calpain and ubiquitin-proteasome system (UPS) and could stabilize the mutant proteins at equal amounts compared with the wild-type protein. In summary, we describe evidence that mutations in PKP2 produce unstable proteins *in vitro*, which might be removed rapidly from the cell by proteolytic degradation.

P11.014 CHIP-based sequence analysis of 34 cardiomyopathy genes reveals new genes involved in HCM and DCM and multiple pathogenic mutations in single patients

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Inherited cardiomyopathy is a common cardiac disease with a prevalence of 1:500 for hypertrophic cardiomyopathy (HCM) and 1: 2500 for dilated cardiomyopathy (DCM). The large genetic and clinical heterogeneity and the laborious screening methods, hamper rapid detection of the genetic cause in all cases. Current diagnostic screening solves only 60-70% of the families by testing a limited number of genes and usually stops when a pathogenic mutation is found. However, double pathogenic mutations seem to be present in 5-10% of the familial cases. In order to create a fast, parallel genetic screening pipeline for inherited cardiomyopathy, we designed a resequencing array (Cardio-CHIP) covering 34 genes in duplicate (300Kb). Genes known to be involved in DCM, HCM, non compaction cardiomyopathy, Limb Girdle Muscular Dystrophy and candidate genes based on their presence in the sarcomere and Z-disc were included, as also exons and flanking introns (38bp) covering the heart- and muscle-specific RNA-isoforms were included. The 5'UTR and 3'UTR regions and, for a selection of

genes, the promoter regions were included as well. So far, 250 patients were sequenced for all 34 cardiomyopathy genes. The mutation detection rate is around 99% and about 98% of the novel exonic variants can be confirmed by conventional sequence analysis. In addition to mutations detected in the 13 genes routinely tested for HCM or DCM, we identified mutations in the 21 additional genes, some of which were the first for those new candidate genes. We present several families in which up to 4 pathogenic mutations were identified.

P11.015 Specific missense mutations affecting the N-terminal region of NIPBL almost completely abolish adherin complex formation with the cohesin-associated protein MAU-2

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Cornelia de Lange syndrome (CdLS) is a dominantly inherited genetic disorder. The phenotype is characterized by typical facial features, upper limb defects, growth and mental retardation. Mutations in the cohesin associated factor NIPBL or the highly conserved structural components of the cohesin ring SMC1A and SMC3 have been identified in about 60% of the patients.

NIPBL directly interacts with MAU-2 to form a heterodimeric complex called adherin which regulates the loading and unloading of cohesin onto chromatin.

Using two hybrid-based techniques we could narrow down the critical region for this heterodimerization to a stretch of 40 amino acids within the N-terminus of MAU-2 and the N-terminal region of NIPBL. Sequencing analysis of *NIPBL* in a cohort of about 100 patients with CdLS, identified six new missense mutations affecting this region.

Reporter gene assays of these six as well as two further mutations could show that missense mutations G15R and P29Q almost completely abolish the NIPBL-MAU-2 complex formation. Whereas mutations S111T, A179T, P192L, D246G, L254V and S262A do not alter heterodimerization.

While no detailed clinical data was available for patient P29Q, patient G15R is severely mentally retarded, shows a typical CdLS phenotype without any abnormalities of the limbs.

Our data show for the first time that specific missense mutations affecting the N-terminal region of NIPBL can almost completely abolish adherin complex formation. Whether these mutations result in an altered cohesion loading or unloading to distinct gene loci which maybe modify the expression of specific genes is currently under investigation.

P11.016 Centaurin- α 2 and tubulin interaction increases microtubule stability

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Centaurin- α 2 is constituted by an Arf-Gap zinc binding domain and two PH domains. In EGF stimulated cells, centaurin- α 2 localizes at plasma membrane through PIP2/PIP3 binding and it promotes the inactivation of Arf6, involved in intracellular vesicular trafficking and in cytoskeletal rearrangement. With the final aim of elucidating the centaurin- α 2 molecular pathways and its biological role/s, we searched for centaurin- α 2 interacting proteins by an yeast two-hybrid assay: our studies evidenced the interaction between centaurin- α 2 and tubulin β , confirmed by coimmunoprecipitation experiments. Confocal microscopy analysis allowed us to co-localize centaurin- α 2 with microtubules. By western blotting analyses we found that centaurin- α 2 is preferentially associated with polymerized fraction of tubulin and remained associated with microtubules resistant to cold or/and nocodazol treatment. Moreover in cell transfected with centaurin- α 2, we observed an increase of acetylated microtubules (MT), a well known marker of stable MT, suggesting that centaurin- α 2 increases the stability of MT.

We are going to define the centaurin- α 2 domain/s interacting with tubulin β by β -galactosidase assay and immunofluorescence experiments. We will check by live imaging the co-localization of centaurin- α 2 with tubulin β during its translocation from cytosol to membrane, by means of GFP-centaurin- α 2 transfection following EGF stimulation. Our findings allow us to speculate that centaurin- α 2 can move to plasma mem-

brane through microtubule anchoring and that this protein could act as agent stabilizing microtubules.

P11.017 Heterozygous 5p13.3-13.2 deletion in a patient with type I Chiari malformation and bilateral Duane retraction syndrome

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The authors used high-resolution array-based comparative genome hybridization (CGH) analysis to characterize the ~2.2 million-bp deletion on chromosome 5 that accounts for this syndromic clinical presentation. Genetic analysis using array-based CGH revealed a deletion affecting multiple genes at the 5p13.3-13.2 locus. Quantitative real-time polymerase chain reaction (RT-PCR) on genomic DNA confirmed this genomic lesion.

A syndrome combining features of Chiari malformation and bilateral Duane's retraction syndrome can be added to the group of entities that result from deleterious genetic variants involving a group of genes and possibly producing contiguous gene syndrome. The deletion detected in our patient can be easily detected using either array-based chromosomal analysis or quantitative RT-PCR.

P11.018 SOLiD™ ChIP-seq kit for ChIP and ChIP-Sequencing from low cell number samples

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Chromatin Immunoprecipitation (ChIP) assay is the widely used and a powerful method to identify regions of the genome associated with specific proteins. Combined with massively parallel next-gen DNA sequencing technology, ChIP-sequencing provides a high resolution digital solution for genome-wide survey of protein-DNA interactions.

We developed a SOLiD ChIP-seq kit, which offers an optimized ChIP workflow and an efficient ChIP-seq library construction from low cell number samples. ChIP procedure is usually laborious, time consuming, and requires large starting cell numbers. We use MAGnify ChIP System, which is suitable for fast enrichment of chromatin complexes and DNA recovery, for ChIP workflow in the kit. It is able to use less than 300,000 cells for ChIP thus preserving precious samples such as primary cells and biopsies. This new approach allows faster throughput to investigate different chromatin and transcription time-course events as well as enable antibody screening to determine ChIP compatibility. In addition, we develop a sensitive ChIP-seq library construction procedure which enables users to construct a complex library using as low as 1 ng ChIP DNA. Combining with SOLiD's high sequencing throughput, SOLiD ChIP-seq offers a highly sensitive, hypothesis-neutral approach to accurately characterize protein-DNA interactions at genome-wide scale.

Using SOLiD ChIP-seq kit, we characterize transcriptionally permissive and repressive histone H3 modifications and transcriptional regulators in the MCF7 cells on the SOLiD platform. The data demonstrate that SOLiD ChIP-seq kit provides a streamlined and reproducible assay for the enrichment of chromatin/protein complexes, DNA recovery using magnetic bead capture technology and construction of sequencing library.

P11.019 Study of gene expression by DNA microarray technology in c-kit mutant mice

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The c-kit gene codifies for a tyrosine-kinase receptor with essential role in development of interstitial Cajal cells (ICC) from gastro-intestinal tract, involved in digestive motility and neurotransmission, but also in GIST (gastro-intestinal tumors) pathogenesis. Interstitial Cajal-like cells (ICLC) have been identified in several extra-digestive organs, their functions being yet unknown.

In order to contribute in understanding the physiology of ICC and ICLC we have performed comparative investigation of gene expression in normal and mutant mice by DNA microarray.

Total RNA was extracted by AllPrep DNA/RNA Mini Kit (Qiagen) from different digestive and extra-digestive organs sampled from control and mutant mice (WBB6F1/J-KitW/KitW-v/J strain) and analyzed by Bioanalyzer and RNA 6000 Nano assay Kit (Agilent Technologies).

DNA microarrays from Whole Mouse Genome Microarray Kit (Agilent Technologies) hybridized and scanned by Agilent DNA Microarray Scanner were analyzed by Feature Extraction 5.1.1. and GeneSpring GX 10. Expression Analysis Software (Agilent Technologies).

DNA microarray analysis showed differential expression (>2 fold) of more than 3000 genes in mutant versus control mice. Thus, genes involved in apoptosis, intra-cellular transport and inter-cellular communication (such as Wnk1, Sema5a, Nf1, Tnfrsf21 genes) were down-regulated. Other genes involved in transcription regulation (such as Eif4a1, Zfp593, Zfp69, Dmbx1), cellular transport and cell junctions (Hbb-b1, Abi2, CaCng8), signal transduction and metabolic process regulation (Calcr, Cyp3a44, Mcpt1 genes) were up-regulated in mutant versus control mice. Some of these genes may become candidate biomarkers for studying ICC and associated pathology in humans after validation by RT-qPCR.

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P11.020 Increased sensitivity of copy number variation using a new high density array CGH platform.

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Array CGH methods are widely used to investigate DNA copy number variation (CNV) associated with complex disorders. Disease-association studies have become increasingly focused on CNVs, and recent reports show links between CNVs and schizophrenia (Stefansson 2008; Stone 2008), autism (Sebat 2007; Marshall 2008; Glessner 2009), and cancer. More recently, the focus of CNV research has migrated to detection of rare variants, with an allelic frequency of less than 5% (Conrad 2009, Manolio 2009). In an effort to increase detection of rare variants as well as more common CNVs, we sought to develop the highest density oligo array available for CNV detection, as well as a more sensitive algorithm for CNV detection on NimbleGen arrays.

The NimbleGen 2.1M and 3x720K CNV arrays contain empirically optimized probes, with the most comprehensive collection of targeted regions available, including Asian population-specific CNV regions. Utilization of the CNV arrays enabled detection of several hundred CNVs per individual. When compared to whole-genome tiling arrays, 2- to 3-fold more CNVs were detected with the CNV arrays. The NimbleGen CNV arrays detected 300% and 245% more CNVs, respectively, and the higher probe density allowed 2-fold higher detection of CNVs shorter than 1 kb when compared to competitor arrays. The increased sensitivity of CNV detection was aided by an improved segmentation algorithm (segMNT), included in NimbleScan v2.6 software. A comparison between the segMNT v1.1 and segMNT v1.2 algorithms shows increased sensitivity in detection of CNVs resulting in about 2-fold more CNVs detected per sample with segMNT v1.2.

P11.021** Phenotypic severity of CRLF1 associated disorders depends on the secretion of the mutated protein

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Crisponi syndrome (CS) and Cold-induced sweating syndrome type 1 (CISS1), are disorders caused by mutations in the cytokine receptor-like factor 1 (*CRLF1*) gene. The two syndromes share some clinical characteristics, including dysmorphic features, scoliosis and cold induced sweating, while Crisponi patients usually show a severe clinical course in infancy involving hyperthermia, muscular contractions and feeding difficulties.

To evaluate a potential genotype/phenotype correlation and whether CS and CISS1 represent two allelic diseases or manifestations at different stages of the same disorder, we performed a detailed analysis of the clinical phenotype of 19 patients carrying mutations in *CRLF1*. 14 of these patients were originally classified as Crisponi syndrome and 5 as Cold induced sweating syndrome type 1.

We studied the functional significance of the mutations by site directed mutagenesis and analysis of the mutant constructs after transfection. We found that phenotypic severity of the two *CRLF1* associated disorders does not depend on the different type/localization of mutation but on altered kinetics of secretion of the mutated *CRLF1* protein.

A lacked, delayed or partial *CRLF1* secretion was associated with a severe clinical phenotype, while a full secretion was associated with a milder clinical phenotype.

We therefore propose a novel classification of patients with *CRLF1* mutations depending on the secretion of the mutated protein.

P11.022 Identification of cryptic recombination signal sequences containing CpG sites

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It is known that inhibition of interactions between RAG1/2 proteins and their DNA target sites *in vitro* can be caused by mCpG sites located inside target sites. For example, methylation at the third cytosine of heptamer on the top strand responsible for direct inhibition DNA cleavage by RAG1/2. By contrast, mCpG sites at other positions block RAG1/2-mediated cleavage if mCpG interact with methyl-CpG binding-domain proteins.

In this study we analyzed nucleotide composition of 5649 human cryptic recombination signal sequences (cRSS) which supposedly have high recombination potential (their heptamer/nonamer sequences corresponded to CACAGTG/ACAAAAAACC structure or differed from them for 1-2 non functional nucleotides). These cRSS previously were found by us in the human genome outside Ig and TCR loci. We consider, that RAG1/2 theoretically can interact with such motives and mediate instability of human genome when V(D)J-recombination system gets out of control. We have found CpG sites in 1513 (27%) cRSS (697 of such cRSS are located in protein-coding genes). 1245, 180, 87 and 1 cRSS have 1, 2, 3 and 4 CpG, respectively. Methylation at the third cytosine of the heptamer can be possible at 221 cRSS. We found that 26%, 69%, 5% CpG are located in heptamers, spacers, nonamers of cRSS, respectively. CpG frequency in 23cRSS is 3.3-fold bigger than in 12cRSS (cRSS with 23 bp and 12 bp spacers, respectively). We propose that *in vivo* when V(D)J-recombination system gets out of control 1513 cRSS containing mCpG will be less effective interact with RAG1/2 than 4136 cRSS without mCpG.

P11.023 Development of a simplified and sensitive microarray-based method for mutation detection with optimised probes for cystic fibrosis

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Oligonucleotide microarrays represent a versatile platform which allows the rapid and cost effective screening of a large number of mutations and sequence variations in specific parts of genomes. Microarray

techniques are being continuously improved to increase the reliability and precision of this method[1]. Here we present the development of an improved mutation detection method using a pool of 40 mutations of the cystic fibrosis transmembrane conductance regulator gene (CFTR) that include the 21 most frequent in the Caucasian UK population. An extensive oligonucleotide design was produced by the IsoDice software and utilized in order to identify probes sets with the highest sensitivity and specificity. This design study allowed us to address, investigate and identify many factors that affect the hybridization kinetics of oligonucleotides. A direct-hybridization gain-of-signal approach[2] allowed the selection of probe pairs for each mutation, working optimally under one single hybridization condition. Testing of the oligonucleotide pairs on different microarray platforms showed the robustness and reliability of the method, showing conserved discrimination between wild type and mutant genotypes. IsoDice and the hybridization protocol were applied to 13 polymorphisms linked to cardio vascular diseases and optimal probe pairs were successfully selected. We have shown a cost effective, simplified and comprehensive CFTR genotyping method on microarrays that can be readily expanded to include additional mutations, and adapted as a diagnostic tool for other diseases.

P11.024 Association of Xq28 recurrent duplication with Dandy-Walker malformation: a putative role for filamin A as predisposing factor.

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The Dandy-Walker malformation is considered as a continuum of posterior fossa anomalies comprising from the classical malformation to megacisterna magna of unknown etiology.

Three patients of the same family, two brothers and one maternal nephew, with mild-moderate mental retardation, and Dandy-Walker malformation in two of them, were investigated by array-CGH.

A duplication of nearly 0.3 Mb was observed at Xq28 between positions 153.2 to 153.5Mb, that perfectly overlaps with the recently described copy-number gain with two, three or five copies of the genes annotated. Our results fit well with the suggestion that the copy number correlates with the severity of clinical features, where GDI1 is the most likely candidate gene in impaired cognition. However, the Dandy-Walker malformation or megacisterna magna, clearly associated to this CNV in four different families, is better correlated with FLNA copy number. Altogether, it is present in 4/8 patients with two copies and in 2/2 patients with three copies of this gene.

At present the mechanism(s) underlying Dandy-Walker malformation remains unknown. A pathogenic theory holds that the primary defect is vermis hypoplasia due to an inhibition of cell migration from rhombencephalon. Filamin A, a widely expressed protein that regulates reorganization of the actin cytoskeleton, is required for locomotion of many cell types. Mutations in this gene lead to a broad range of congenital malformations, including a localized neuronal migration disorder. Hence, deregulation of neuronal migration favoured by an increased expression of filamin A would be in good accordance with a predisposition to this abnormality.

P11.025 THE INSILICO DATABASE: AN EFFICIENT STARTING POINT FOR GENOMIC DATA ANALYSIS

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There is a large and rapidly growing amount of genomic data freely available in the public domain, but this data is often in a raw form and requires tedious and error-prone retrieval and compilation before it can be used in analysis tools. The In Silico database allows fast data import and collaborative curation from the public databases. Raw biological data are pre-processed uniformly and the biological sample description is compiled into a thesaurus form. These features allow the efficient aggregation of the data as well as the sample annotation from multiple studies for joint analysis.

The genomic datasets compiled in the In Silico database can then be seamlessly queried and retrieved along with the biological information for export to analysis platforms such as R/bioconductor and GenePattern.

We compare and contrast the functionality with other similar systems and outline the key features of the systems. We present a case study

over the term «age» across multiple human expression datasets. The system is available at <http://insilico.ulb.ac.be>

P11.026 Diagnostic Mutation Database, Repository of UK Molecular Diagnostic mutation data

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The Diagnostic Mutation Database (DMuDB) is a repository for sharing variant data among UK molecular diagnostic laboratories, developed and curated by the National Genetics Reference Laboratory (NGRL) in Manchester, UK. The database is accessed through a secure website. Data are anonymised and are shared according to the Data Protection Act 1998. They are uploaded manually (one referral at a time) by laboratories when diagnostic reports are issued, or in bulk (more than one referral at a time) by the database curator. DMuDB variant names are based on HGVS nomenclature but it can also store other nomenclature systems. NGRL has also developed the Universal Browser to view data from DMuDB, dbSNP, LSDBs and other data sources graphically in an integrated display.

DMuDB was first released in 2005. It is centred on patient referrals and presently holds 6,900 referrals which contain more than 13,000 individual variants in 30 genes. There are 228 active users from 39 diagnostic laboratories. BRCA variants (~9200) and HNPCC variants (~1100) currently predominate but the range of data is expanding, for example we are currently expanding DMuDB to support EGFR mutation, treatment and response to treatment information. Users outside of UK laboratories can enquire about variants in DMuDB, and will be put in touch with a relevant submitting laboratory when appropriate. We are also developing partnerships, e.g. with ENIGMA group for BRCA data and InSIGHT for Lynch Syndrome data, so that DMuDB data can be submitted to publically accessible databases.

P11.027 A novel mutation on COCH gene in an Italian family.

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Hearing loss is the most common form of sensory impairment, affecting millions of individuals worldwide. Pre-lingual non-syndromic hearing loss is present in 1 on 700 children and it is thought that in half of the cases the hearing impairment is caused by genetic factors. Progressive late onset hearing loss is much more frequent than pre-lingual deafness. Most cases are caused by interactions of non-genetic (infection, acoustic trauma and ototoxic drugs) and predisposing genetic factors. We analysed an Italian family with a bilateral sensorineural hearing loss attended in many family members and inherited in an autosomal dominant manner.

The subjects were genotyped with the SNPs array using the HumanCNV370-Duo platform (Illumina, San Diego, California) according to manufacturer's protocol. Then we performed parametric linkage analysis using Merlin which identified a close to significant 40 Mb locus on chromosome 14.

The only gene known to cause deafness in this region was COCH. The sequence of all exons and the flanking regions of the gene allowed us to identify a novel mutation A487P. This mutation was found in all of the affected family members and was not found in healthy family members or in 100 healthy controls.

P11.028** Diamond-Blackfan Anemia associated mutations in Ribosomal Protein S19 impair its binding to its own mRNA

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Diamond Blackfan Anemia (DBA) is a congenital pure red cell aplasia (OMIM 205900) typically presenting within the first year of life. The disease is also associated with growth retardation, congenital malformations and heterozygous mutations in a subset of ribosomal protein genes. Heterozygous mutations in the ribosomal protein S19 (RPS19) gene are observed in approximately 25% of DBA patients. The mutations result in perturbed rRNA processing and impaired ribosomal subunit formation. The mechanisms whereby disruption of ribosome biogenesis results in anemia remain to be defined. We hypothesized that RPS19 interacts with its own mRNA as part of a regulatory mechanism.

We used electrophoretic mobility shift assays (EMSA) and filter binding experiments to address whether rRPS19 binds to the 5' untranslated region (5' UTR) of its own mRNA and whether this interaction is of biological significance. We show that RPS19 binds specifically to the 5' UTR of its own mRNA with an equilibrium binding constant (K_D) of 4.1 ± 1.9 nM. The binding is sequence and structure dependant as mutations introduced into the 5'UTR lower RPS19's affinity to the substrate. Additionally, we investigated the mRNA binding properties of two mutant RPS19 proteins (W52R and R62W) identified in DBA patients. We observed a significant increase in K_D for both proteins (16.1 ± 2.1 and 14.5 ± 4.9 nM, respectively), indicating a reduced RNA binding capability ($p < 0.05$). We suggest that the binding of RPS19 to its mRNA has a regulatory function and hypothesize that the weaker RNA binding of mutant rRPS19 may have implications for the pathophysiological mechanisms in DBA.

P11.029** Tissue-specific conserved coexpression for disease gene prediction and hypotheses generation

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Even considering recent technological and methodological advances, such as next generation sequencing and genome-wide association studies, the identification of genes involved in human hereditary disease remains a demanding task that can be significantly aided by computational predictions.

We discuss a method based on high-throughput microarray expression data that uses the conservation of coexpression as a powerful filter for biological significance and allows to specifically focus on tissue-specific relationships between disease and candidate genes.

The most important novelty of our approach, the tissue-specificity that we show to be highly complementary to multi-tissue coexpression, has allowed to identify novel high-confidence candidates for several genetic diseases. Moreover, disease gene prediction via tissue-specific conserved coexpression can additionally generate meaningful hypotheses about functions of candidate genes and biological processes underlying disease. Notably, these hypotheses can be important also for cases other than disease conditions, and have provided us with several promising novel candidates for pluripotency.

Through an explicit integration of phenomics, in particular the concept of phenotype similarity, we can apply our method also to disease phenotypes with so far unknown molecular basis. We present a user-friendly web tool for custom analysis, discuss the results obtained, and describe a case study that confirms USP9X as a particularly interesting candidate for X-linked mental retardation. The latter is also a proof of concept that our method can be efficiently integrated with deep sequencing to provide high-confidence candidate genes.

P11.030 Targeted Diagnostic Sequencing of patients with genetic disorders

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It has been estimated that 5% of live births are affected by a genetic disorder that will manifest before adulthood. These disorders can have a significant, lifelong impact on affected individuals and their families, along with major economic burdens on society in general. Being able to provide a rapid and accurate diagnosis is beneficial for effective treatment of patients as well as counselling of family members.

Recent developments in DNA sequencing technologies have great potential for advancing genetic research and diagnosis. Sequencing the entire genome of an individual however is still prohibitively expensive for the majority of research and diagnostic laboratories, and the amount of data that needs to be analysed will in the majority of cases preclude a rapid result being provided to the clinician.

We have developed a technique that captures multiple genomic regions of interest for sequencing. It is based on the immobilization of target DNA to small nylon filters, which are then used to capture sequences of interest from fragmented and bar-coded genomic DNA. We have trialled this technique using PCR products and BAC sequences as capture material and in each instance have found >10,000 fold enrichment for the DNA sequences targeted. We are currently developing several specific target mixes, particularly focussing on genes involved in Disorders of Sex Development (DSD) and Premature Ovarian Fail-

ure (POF). This approach has the potential to allow the rapid and cost-effective diagnosis of a range of genetic disorders.

P11.031 Automatically extracting implicit knowledge from literature for biological data interpretation and function prediction.

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The amount of scientific knowledge needed to generate hypotheses, conduct experiments and interpret results in the biomedical domain is increasing rapidly, and reading all the relevant literature is already infeasible for any human. This is especially true when studies become more and more interdisciplinary. To tackle this problem we developed techniques that generate „concept profiles“ from literature, an approach we call „Biosemantics“ (www.biosemantics.org).

Biosemantics tools include Anni, a multipurpose text-mining tool that can be used to analyse gene expression profiling data, and Nermal. Nermal predicts Protein-Protein Interactions (PPIs) based on the similarity of the context in which the individual proteins appear in literature. It outperforms previously developed PPI prediction algorithms that rely on the conjunction of two protein names in MEDLINE abstracts. We show significant increases in coverage (76% vs. 32%) and sensitivity (66% vs. 41% at 95% specificity) for the prediction of PPIs currently archived in six PPI databases. The practical value of the method for discovery of novel PPIs was illustrated by the experimental confirmation of the predicted interaction between CAPN3 and PARVB, based on shared concepts such as Z-disc, dysferlin and alpha-actinin. Using similar Biosemantics approaches we are currently studying the predictive power of our methods for relationships between genes and phenotypes (e.g. diseases). Using roll back analysis we simulate the prediction of a disease-causing gene before publication of the actual landmark paper describing the connection between the gene and the disease.

P11.032 Improved detection of cell-free methylated DNA in plasma

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Detection of cell-free methylated DNA in plasma is a promising tool for tumour diagnosis and monitoring. Due to the very low amount of cell-free DNA in plasma, sensitivity of the detection methods are of utmost importance. The vast majority of currently available methods for analysing DNA methylation are based on bisulphite-mediated deamination of cytosine. Cytosine is rapidly converted to uracil during bisulphite-treatment, whereas 5-methylcytosine is only slowly converted. Hence, bisulphite-treatment converts an epigenetic modification into a difference in sequence, amenable to analysis either by sequencing or PCR based methods. However, the recovery of bisulphite-converted DNA is very poor. Here we introduce an alternative method for the crucial steps of bisulphite removal and desulphonation, vastly improving recovery, especially for specimens with low levels of methylated DNA (table 1).

Table 1 Detection frequency of methylated copies

| Methylated genome copies | 1.25 | 2.5 | 5 | 10 |
|--------------------------|------|-----|-----|-----|
| Detection frequency* | 31 % | 45% | 66% | 86% |

*calculated as the percentage of positive results of 18 markers analysed 6 times each.

The method is based on an accelerated deamination step and magnetic silica purification of DNA in combination with a first round of PCR amplifying 18 methylated markers concurrently, followed by individual detection of the 18 methylated markers by real-time PCR. This method allows low levels of DNA to be easily and reliably analysed, a prerequisite for the clinical usefulness of cell-free methylated DNA detection in plasma.

P11.033 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 potentially used as diagnostic or prognostic biomarkers. Over 100 methylated loci and genes were previously known in non-small cell lung cancer (NSCLC). We've focused our methylation study on early-stage NSCLC.

We've analysed stage I NSCLC samples from 49 patients together with 19 matching macroscopically cancer-free control samples for methylation changes with Infinium HumanMethylation27 beadarrays (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 264 genes hyper- and 423 genes hypomethylated in NSCLC (Bonferroni corrected, $p < 0.01$). From these genes, 113 genes were differentially methylated in adenocarcinoma and 194 genes differentially methylated in squamous cell carcinoma (Bonferroni corrected, $p < 0.01$). Five genes showed significant differences in methylation level in the patients with up to 1 years survival, compared to patients with 5 and more years survival (Wilcoxon rank-sum test, $p < 0.05$). At the time of abstract submission, correlation analysis with gene expression data of the differentially methylated genes is underway.

The methylation markers found will need a further validation. To our surprise, the number of hypomethylated genes in stage I NSCLC was higher compared to the number of hypermethylated genes.

P11.034 Quantitative one-step DNA methylation analysis using native genomic DNA as template

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During the last few years, analysis of DNA methylation has become a standard procedure in both research and diagnostics. Analysis of DNA methylation currently requires multi-step procedures that are either based on conversion of unmethylated cytosines by bisulfite or on methylation-sensitive endonucleases. In the present study, we investigated the potential of a novel one-step approach we refer to as quantitative one-step DNA methylation analysis (qOSMA). The assay is based on the combination of methylation-sensitive FastDigest® endonuclease digestion and quantitative PCR (qPCR) in a single reaction, with reaction conditions providing DNA digestion in a first step, followed by endonuclease inactivation and qPCR. The degree of DNA methylation is determined by comparing the quantification cycles (C_q) of reactions containing either a methylation-sensitive endonuclease or a sham mixture with no endonuclease. Using our novel approach, we correctly diagnosed the imprinting disorders Prader-Willi syndrome (PWS) and Angelman syndrome (AS) in 35 individuals by determining methylation levels of the SNRPN promoter. Control reactions interrogating an unmethylated locus detected possible endonuclease inhibitors and were simultaneously used for copy number assessment by means of standard qPCR with the delta ΔC_q method. In addition, we propose a model for digestion bias correction, which significantly increases assay accuracy and allows analysis of DNA samples with decreased digestibility like they occur in retrospective studies. Our procedure strongly reduces both hands-on time and the risk of handling and pipetting errors and allows DNA methylation analysis to be completed in less than 90 minutes after DNA extraction, predestining the assay for high-throughput analyses.

P11.035 Deep sequencing analysis of pre-mRNA splicing in the human DMD gene.

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Duchenne muscular dystrophy (DMD) is a recessive X-linked disorder and is caused by mutations in the *DMD* gene that disrupt the open reading frame, resulting in a truncated and non-functional protein. The absence of the dystrophin protein causes a progressive neuromuscular degeneration, which develops into complete loss of muscle fibers and muscle function. A possible conversion in a milder phenotype is

achieved by an innovative approach which restores the semi-functional dystrophin proteins, using antisense oligonucleotides (AONs). This strategy has been successfully used for single and double exons skipping, but it is not full efficient for multiple exons, although it might be improved by an increased acknowledge of the splicing of *DMD* gene. We present a first preliminary study of a complete transcriptome analysis of the human *DMD* gene, characterizing all encoding transcripts, splice junctions, the order of intron removal and alternative splicing. To accomplish these aims, we performed deep sequencing of entire 2.4 Mb *DMD* transcript (Solexa Illumina), after the capture of the fragmented reverse transcripts by hybridization to oligonucleotide probes in solution (SureSelect system from Agilent). To target all introns, 79 exons and the 8 alternative transcripts, we created a biotinylated library, which minimally overlap to the complete coding and non-coding regions. Paired end sequencing will allow confirmation of the enriched *DMD* gene. The enormous amount of processed data can support a different correlation between the transcripts abundance and the removed introns stage, providing a novel insight into the mechanism and the regulation of the splicing of *DMD* gene.

P11.036 A functional link of DYT1 and DYT6 dystonia: Repression of TOR1A (DYT1) gene expression by the transcription factor activity of THAP1 (DYT6)**

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Dystonia is clinically characterized by involuntary twisting, repetitive movements and abnormal postures. The monogenic forms of primary (isolated) dystonia are associated with mutations in two genes: i) mutations in the *TOR1A* gene cause DYT1 dystonia; ii) mutations in the recently identified *THAP1* gene lead to the phenotypically similar DYT6 dystonia. The THAP1 protein is a sequence-specific DNA-binding factor involved in transcriptional regulation, with a characteristic THAP zinc-finger domain at the N-terminus.

To illuminate the molecular association between DYT1 and DYT6 dystonia, we performed luciferase reporter gene assays. For this, the *TOR1A* core promoter was narrowed down to a stretch of 200 bp and inserted into the pGL4 luciferase reporter plasmid. Co-transfection of the *TOR1A* core promoter with a THAP1-expression plasmid decreases *TOR1A* promoter activity in a concentration-dependent manner. In addition, DYT6 dystonia-associated mutant THAP1 proteins generated via site directed *in-vitro* mutagenesis showed impaired THAP1-mediated repression of *TOR1A*. This was confirmed *in vivo* by measuring *TOR1A* expression in primary fibroblasts from THAP1 mutation carriers by quantitative PCR, where mutation carriers showed a two-fold increase in *DYT1* expression as compared to controls. Furthermore, we performed chromatin immunoprecipitation (ChIP) in human neuroblastoma cells (SH-SY5Y) to prove that THAP1 binds to the *TOR1A* promoter.

Our data collectively demonstrate that THAP1 regulates the transcription of *TOR1A*, establishing transcriptional dysregulation as a cause of dystonia. Further, we provide evidence that the molecular pathways underlying DYT1 and DYT6 dystonia are linked with a central role of TorsinA in the pathogenesis of dystonia.

P11.037 A novel approach for denaturing high-resolution DNA electrophoresis of MLPA and STR-Typing samples in microfluidic chips

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The Agilent 2100 Bioanalyzer instrument platform is designed for performing fast electrophoretic analysis of DNA, RNA and proteins based on microfluidic chip technology. It is widely used for the analysis of PCR products and restriction digests. However, the resolution of the current DNA assays is not sufficient to separate DNA fragments generated in applications like e.g. STR-typing, AFLP and MLPA. As these methods require a very high resolving power in the electrophoresis involving denaturing conditions, usually capillary-based DNA sequencers are used to separate the DNA fragments. However, due to a growing number of new applications, there is a strong demand for an alternative separation platform that is fast, flexible and easy to use.

Here we describe a novel Bioanalyzer assay prototype that is able to perform fast, sensitive, high resolution separation of labeled DNA fragments on a modified Agilent 2100 Bioanalyzer instrument. A new denaturing gel matrix has been developed that enables the separation of DNA fragments between 10 and 550 bp with a resolution approaching 2 basepairs. By the use of an internal size standard very precise sizing results can be achieved (standard deviation approx. 0.1-0.2 bp). The significantly increased resolution of the new assay together with the possibility to use an internal size standard enables applications like STR-typing and MLPA analysis on the Bioanalyzer.

P11.038 GENCODE: Creating the Human Reference Geneset

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The Encyclopedia of DNA Elements (ENCODE) project aims to identify all functional elements in the human genome. Having the best possible annotation of coding and non-coding genes and pseudogenes is an essential part of achieving this goal. The GENCODE consortium are producing the reference human gene set which will provide the basis for subsequent analysis. This is being achieved using a combination of manual annotation, computational prediction and experimental validation. The manual gene annotation, while requiring support from transcript or protein evidence, is both informed by and QC tested against computational gene predictions of alternatively spliced transcripts, supported introns, U12 introns, coding exons and pseudogenes to ensure high sensitivity and specificity. Manually annotated gene models are then validated by RT-PCR and sequencing.

To achieve a more complete coverage of loci and alternative splice variants (including the complete set of Consensus Coding Sequences (CCDS)), manual annotation from the HAVANA group has been merged with automated gene predictions from Ensembl. A non-redundant set of transcripts with confidence levels to clearly indicate their source has been created and is available via the Ensembl and UCSC genome browsers and DAS servers.

In addition to the ENCODE project, both the 1000 Genomes Project and the International Cancer Genome Consortium have adopted GENCODE as the reference gene set for analysis.

P11.039 Gene expression analysis under the action of Selank and its fragments

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Selank is a synthetic peptide which consists tuftsin (Thr-Lys-Pro-Arg) and C terminal Pro-Gly-Pro peptide. Selank has a nootropic and anxiolytic effects, and exhibits an antiviral effect.

To study the effect single and course intranasal Selank administration on gene expression we have analyzed the expression profiles of 12000 genes in rat hippocampus. Single and course administration of Selank caused a more than 2.5-fold change in the expression of 18 and 15 genes, respectively. Both single and course administration of Selank caused change in the expression of five genes: *Actn1*, *Cx3cr1*, *Fgf7*, *Ptpn2* and *Xtrp1*. Also it was shown that the effect of Selank on the expression of these genes in the spleen is much stronger than in the hippocampus. The most significant increase was observed for three genes (*Ptpn2*, *Actn1* and *Cx3cr1*) after a single administration of Selank.

For a more detailed study the action of Selank and its fragments - Gly-Pro, Arg-Pro-Gly-Pro and tuftsin - on the gene expression analyzed the expression of 84 genes involved in processes of inflammation in the mouse spleen 6 and 24 hours after single intraperitoneal injection of peptides. The result was a significant change in the expression of 34

genes. In most cases the response was observed 6 hours after of peptides injection. Also should be noted that each of the peptides gives an individual picture of changes in the expression of genes under study.

P11.040 Deficiency of Npps attenuates atherosclerosis lesion progression in apoE deficient mice**

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Mutations in *ENPP1* are associated with media calcification in generalized arterial calcification of infancy (GACI). *ENPP1* encodes for Ecto-Nucleotide Pyrophosphatase/ Phosphodiesterase 1 (NPP1), which generates inorganic pyrophosphate, a physiologic inhibitor of hydroxyapatite deposition. The role of NPP1 in atherosclerosis is not known. *Npps/apoE* double knockout mice were generated by cross-breeding *Npps*-deficient *ttw/ttw* mice with *apoE* null mice and were fed a high fat/ high cholesterol diet. Atherosclerotic lesion area and calcification were examined. In 28 week old *Npps^{+/+}apoE^{-/-}* and *Npps^{-/-}apoE^{-/-}* mice atherosclerotic lesion size was $64\% \pm 20\%$ and $59\% \pm 22\%$ smaller than in *Npps^{+/+}apoE^{-/-}* mice ($p<0.05$). However, there was no significant difference in plaque calcification, whereas only *Npps^{+/+}apoE^{-/-}* mice developed media calcification. In aortic smooth muscle cells isolated from *Npps^{+/+}apoE^{-/-}* and *Npps^{-/-}apoE^{-/-}* mice expression of the pro-atherogenic mediator osteopontin (Opn) was $14\% \pm 8\%$ and $30\% \pm 7\%$ less than in *Npps^{+/+}apoE^{-/-}* mice ($p<0.05$). Opn was previously shown to be a chemoattractant for monocytes and macrophages, which play a key role in early stages of atherosclerosis, i.e. monocyte transendothelial migration, differentiation into macrophages and macrophage uptake of modified lipoprotein. The present study shows that a truncating mutation on one or both alleles of *Npps* reduces atherogenesis in a mouse model of atherosclerosis. We found evidence that decreased plaque formation in mice carrying mutations in *Npps* was mediated through decreased Opn expression levels. We conclude that although mutations on both *ENPP1* alleles lead to GACI, heterozygosity of the *ENPP1* locus may be protective against atherosclerosis.

P11.041 High Throughput Targeted Resequencing of the Human Exome Using Solution-Phase Sequence Capture

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Human exome resequencing is an efficient way to identify functional genomic variation underlying both Mendelian and complex genetic diseases (e.g. cancer, diabetes and Alzheimer's disease). The term "exome" refers to all the exons in the human genome, which are arguably the most functionally relevant 1% of the genome. Currently over 80% of all known mutations for human genetic diseases reside in coding exons and splicing sites, making resequencing this 1% portion of the genome highly attractive. Here we present a high throughput method to capture ~36Mb of target sequence, encompassing the latest definition of the human exome, in a single tube with 2.1 million distinct DNA oligonucleotide probes. Probe design, library construction, library amplification, hybridization and washing have been optimized to deliver highly uniform, specific, and reproducible enrichment of the human exome. We demonstrate that deep coverage of the exome and accurate detection of variants can be achieved using this technology at only a fraction of the cost of whole genome sequencing.

P11.042 Comparison of gene expression profile during normal and FSHD myogenesis support a defect in early stages of the disease**

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disorder mainly associated with a contraction of the subtelomeric repeat D4Z4 on chromosome 4. The D4Z4 copy number is highly polymorphic in normal individuals ranging between 11-150 copies, whereas almost all FSHD patients (FSHD-1) carry fewer than 11 re-

peats. Nevertheless there is a subgroup of patients presenting clinical signs of disease without D4Z4 contraction (FSHD-2). Current hypothesis on FSHD-1 pathogenesis foresee the presence of an epigenetic molecular mechanism, but, how the contraction of D4Z4 array could determine alterations in chromatin structure and trigger transcriptional deregulation of target genes is not clear. Moreover the specific gene(s) responsible for FSHD phenotype have not yet been identified. To clarify some of these aspects, we used the *Human GeneChip Exon 1.0 ST* platform to analyze the global gene expression profiles of FSHD-1, FSHD-2 and controls proliferating myoblasts and the corresponding myotubes. Comparisons of normal and FSHD-1 myoblasts identified a greater number of deregulated genes in comparisons to normal and FSHD-1 myotubes, suggesting a defect in early stages of FSHD-1 differentiation. Moreover, the gene-expression profiles of FSHD-1 and FSHD-2 myoblast exhibited different categories of deregulated genes, demonstrating that different molecular mechanisms are responsible of the disease. The obtained results also suggest that miRNAs could be involved in the regulatory network of FSHD. Our approach provided new insights into the molecular mechanism of FSHD, allowing the identification of new candidate genes that may represent potential targets for clinical application.

P11.043 Chronic Obstructive Pulmonary Disease in mice heterozygous for the *Fgf10* gene

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The most important risk factor for the development of Chronic Obstructive Pulmonary Disease (COPD) is cigarette smoking, but only a minority of smokers will develop COPD, which implies that genetic factors may be involved. Familial aggregation of lung function has been demonstrated and COPD also demonstrates clustering within families, which supports a genetic contribution to pulmonary function. Genetic studies in human and animal models suggest that genes originally associated with lung developmental processes could be implicated in COPD. Fibroblast growth factor-10 (FGF10) signalling through the FGFR2b receptor is required for the development of many branched organs including lungs, thyroid, pituitary, lacrimal, and salivary glands. The most compelling evidence that the FGF10-FGFR2b pathway is essential for lung development was first demonstrated by the profound disruption of branching morphogenesis in mice with either disrupted *FGF10* or *FGFR2b* gene.

We hypothesized that haploinsufficiency of *Fgf10* influence lung function and lead to the development of lung disorders. To examine this hypothesis, we measured the pulmonary functions of mice heterozygous for *Fgf10*. *Fgf10* +/- mice show decreased body and lung weight, although the lung/body weight ratio does not differ from the wild type littermates. *Fgf10* +/- mice exhibit a significant decrease in FEV75, FVC, as well as a decrease in the FEV75/FVC quota when compared to wild type littermates. This would correspond to an airway obstruction in humans. From our results, we suggest that the FGF10 pathway may be highly relevant in search of targets for intervention in obstructive lung disease in humans.

P11.044 microFIND approach for Fluorescence In Situ Hybridization (FISH) detection of genetic lesions from scarce cell samples in hematological malignancies

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Despite improvements in sensitivity for the identification of chromosomal aberrations by fluorescence based methods (1, 2), low cell number recovery from pathological samples may make it difficult in diagnostics. Therefore, processing of limited number of cells still represents a technological challenge.

We have developed a microfluidic device (microFINDTM) for collecting living cells for FISH analysis. microFINDTM is a glass slide coated with a nanomaterial (3) that immobilizes cells inside a microchannel: a miniaturized FISH protocol is carried out and the slide evaluated by high resolution fluorescence microscopy.

We validated microFINDTM in samples from patients affected by Mul-

multiple Myeloma (MM), a malignant proliferation of bone marrow plasma cells. Often, cell purification is needed for investigations because of the small percentage of clonal plasma cells present in the bone marrow. We analyzed 4 MM patients: PCs were purified from bone marrow aspirates using MidiMACS system (4) and directly immobilized on microFINDTM.

We compared FISH performance by microFINDTM and standard protocol of specific MM genetic lesions and demonstrated that microFINDTM approach allows FISH analysis of scarce cell samples (down to 1000 plasma cells/μl), with the same reproducibility compared to standard protocol.

Furthermore the assay miniaturization offers a cost saving approach (only 0.3 μl of fluorescent probe/sample) suitable for automation and throughput increase for screenings of MM in a "chip" configuration.

1. Ntouropoulos et al. Br J Cancer. 2008 (99)
2. Medintz et al. Int J Nanomedicine. 2008 (3)
3. Carbone et al. Biomaterials. 2006 (27)
4. Fabris et al. Genes Chromosomes Cancer 2005 (42)

P11.045 Molecular Characterization of FKRP related Limb-Girdle type 2I Muscular Dystrophy

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Limb-Girdle Muscular Dystrophy type 2I (LGMD2I) is an autosomal, recessive, disorder caused by mutations in the FuKutin-Related Protein gene (FKRP). Muscle biopsy extracts from LGMD2I patients show incomplete/aberrant O-glycosylation of the membrane/extracellular-matrix protein alpha-dystroglycan. However, our knowledge of the function of FKRP is limited and the underlying molecular aetiology of LGMD2I is currently unknown.

Here, we provide data on the posttranslational modification and molecular self-interaction of FKRP (495aa). Deglycosylation of FKRP with either PNGaseF or EndoH results in a FKRP MW shift from 58 to 54 kDa. When over expressed in mammalian cells, FKRP assembles into DTT sensitive complexes of 115 -120 kDa that are DTT insensitive upon *in vitro* chemical cross-linking. Pair-wise Yeast 2-Hybrid experiments with FKRP/FKRP in bait/pray combination sustained growth of yeast on selective medium. Immunogold Transmission Electron Microscopy of *rectus femoris* muscle sections show that antibodies to FKRP co-localize with Golgi marker mg160.

We conclude that FKRP in human skeletal muscle, is localized to the the Golgi compartment, close to the Z-lines between the myofibrils. FKRP contains two potential N-glycosylation sites. Both are occupied with high mannose (and/or hybrid) oligosaccharides. FKRP homodimerization, indicated by chemical cross-linking, was confirmed by pair-wise Yeast 2-Hybrid analysis. FKRP involvement in higher MW complexes (>180 kDa) was also observed. The content of these complexes remains to be investigated.

P11.046 SIVA is unlikely to contribute to the pathogenesis of Familial Mediterranean Fever (FMF): A genetic, structural biological and functional study

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Background: Mutations in *MEFV* gene encoding pyrin account for Familial Mediterranean fever (FMF), which is characterized by recurrent, acute and self-limiting attacks of fever. Pyrin plays a major role in the regulation of IL-1 beta secretion and the pathogenesis of FMF. Aim: To explore the potential contribution of the interaction between

pyrin and the proapoptotic protein Siva-1 to the pathogenesis of FMF. Materials and methods: The three-dimensional model (3-D) model of Siva-1 was created with MODELLER and the Swiss-model automated protein structure homology-modelling server. HEK293 (human embryonic kidney) and THP-1 (human monocytic leukemia) cell lines were used for transfection experiments and apoptosis was measured by FACS-Annexin-PE staining using the Annexin-V kit.

Results: In the 3-D model of Siva-1 we defined three distinct domains. In immunoprecipitation experiments on transfected HEK293 cells, Siva bound both to wild type pyrin and its mutant forms. No differences in rates of apoptosis in THP-1 upon transfection with mutant forms of pyrin were observed. Patients with FMF did not display any mutations in the Siva gene.

Conclusions: The positions of the PRYSPRY C-terminal domain of pyrin, found mutated in FMF patients, are not required for binding to Siva-1. We were not able to demonstrate an increase of Siva-induced apoptosis in the presence of the mutant forms of pyrin. Siva is unlikely to contribute to the pathogenesis of FMF through the postulated molecular mechanism.

P11.047 A missense mutation within the fork-head domain of the FOXG1 gene affects its nuclear localization

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The fork-head box G1 gene (FOGX1, MIM 164874) encodes a transcriptional factor that is critical for forebrain development. It has recently been associated with the congenital variant of Rett syndrome (RTT, OMIM 312750), and so far 17 mutations, including microdeletions, nonsense, missense, splice and frame-shift have been reported. We screened the coding region by direct sequencing in 50 patients affected by postnatal microcephaly, and identified two missense mutations: the c.326C>A (p.L109P) substitution inherited from the healthy father; and the de novo c.730C>T transition, which induces the p.R244C mutation within the DNA-binding fork-head domain. This latter mutation is carried by an 8-year-old girl, who was born at full term with normal weight and height, but relative little head circumference (33 cm, 10th percentile). At last evaluation, she presented with severe microcephaly (<3rd percentile), and MRI showed frontal, gyral simplification without pachygyria, severe hypomyelination, and thin corpus callosum. She was able to walk with aid and combined dyskinetic movement disorders with hand stereotypies, relative good eye contact and communication skills that contrast with severe motor impairment. We constructed a plasmid encoding the mutant FOXG1_p.R244C protein to investigate its subcellular pattern. Immunofluorescence analysis of the wild-type protein revealed a homogeneous nuclear staining excluding the nucleoli, while the mutant showed abnormal nuclear foci in a large proportion of cells, suggesting that its mislocalization may reduce and/or impair target recognition. Molecular analyses are currently in progress to investigate FOXG1 putative targets and to elucidate the molecular pathways involved in the pathophysiology of the disease.

P11.048 Methylation patterns associated with FRA12A

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Dynamic mutations are repetitive sequences that undergo unstable inheritance. Repeat expansion may lead to methylation and transcriptional silencing of associated genes, which is a major cause of human diseases, including cognitive dysfunction. When cells are grown under specific cell-culture conditions they become visible as chromosomal gaps or breaks, called fragile sites. So far, eight rare folate-sensitive fragile sites were cloned, four of which are associated with mental retardation.

Recently we cloned FRA12A and demonstrated that the molecular basis of this rare folate-sensitive fragile site is a CGG-repeat expansion at the 5' end of the *DIP2B* gene, resulting in methylation of its promoter region. The phenotype of FRA12A-carriers varies from severe mental retardation to apparently unaffected. Recurrent miscarriages have also been reported. In all affected patients the repeat was meth-

ylated. In unaffected FRA12A carriers however, the repeat was either methylated or unmethylated. Expression of the *DIP2B* gene was on average higher in unaffected FRA12A carriers compared to affected patients. Unmethylated FRA12A carriers rather showed overexpression of *DIP2B*.

We hypothesized that the difference in expression and phenotypic features could be the result of the varying degree of methylation of CpG-islands between affected patients and unaffected FRA12A carriers. Therefore we are establishing a methylation profile of the CpG-islands surrounding the repeat for all available patients, their family members and a control population. So far we found convincing evidence that at least one CpG-island is less densely methylated in unaffected carriers than in affected carriers, supporting our hypothesis.

P11.049 Validation of two new PCR technologies for diagnostic testing of the Fragile X CGG repeat as well as *FMR1* methylation status.

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Almost all cases of Fragile X syndrome are caused by an expansion of a CGG repeat in the 5' UTR of the *FMR1* gene to more than 200 repeats. Molecular diagnostic testing is currently performed by both PCR of the CGG repeat and Southern blot analysis.

We are validating two new PCR technologies from Asuragen Inc. USA. The first is a kit that supports two PCR configurations: a gene-specific *FMR1* PCR and a CGG repeat primed PCR. With this kit it should be possible to detect the full range of CGG repeats and provide information on the zygosity status in all samples. We tested 75 samples (25 prenatal and 50 postnatal) including full expanded alleles, premutations, intermediates, homozygous normal females and normal males. All results were concordant with previous results. In cases of homozygous normal females, the CGG repeat primed PCR was proven to be very useful and Southern blot analysis is no longer required in these cases.

The second technology we are validating is a prototype PCR method that was developed to detect the *FMR1* methylation status. Currently, the methylation status can only be detected by performing Southern Blot analysis, which is very labor intensive, needs a lot of input, has a low throughput and is not amenable to automation. We have tested both prenatal and postnatal samples to investigate whether this methylation PCR could replace the Southern Blot analysis.

Data from both PCR technologies will be presented in this poster as well as in the Eurogentest Satellite meeting.

P11.050 Easy, accurate genome-wide detection of gene fusions with the SOLiD system using BioScope software

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Gene fusions are implicated in the initiation of tumorigenesis and are important diagnostic/prognostic indicators in leukemia and solid tumors.

The SOLiDTM System 4.0 generates up to 1 billion mapped reads per run, enabling genome-wide hypothesis-free detection of gene fusions. DNA barcoded paired-end reads facilitate cost-effective concurrent sequencing of many samples.

We developed a novel algorithm to detect exon junctions with strand-specific SOLiD system transcriptome sequencing, to predict fusion transcripts and alternative splicing. For paired-end whole transcriptome sequencing experiments, we require two types of evidence; the two tags must map uniquely to two different exons, and a single tag must span the exon junctions. Single-read placement is done with a novel algorithm that allows fast detection of reads split between any pair of exons. Gene fusions are called if there is sufficient non-redundant evidence of both types.

We validated the algorithm with real and simulated data; we detect >80% of simulated human gene fusions at well covered exons.

We sequenced UHR, and we predicted 36 fusions using initial thresholds. We validated these predictions with TaqMan assays. 44% of our predictions were correct, including the three previously annotated gene fusions in UHR; BCR-ABL1, BCAS4-BCAS3 and GAS6-RASA3. We validated an additional 13 novel gene fusions, many of which are putative read-throughs from genes in close proximity. 13 of these gene fusions are also present in the MCF7 breast cancer cell line.

Easy, low-cost genome-wide detection of novel gene fusions allows interrogation of large numbers of samples and discovery of biologically important gene fusions.

P11.051 Using GEN2PHEN software to integrate genotype-to-phenotype data.

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The GEN2PHEN project is developing new software to enable the integration of Genotype-To-Phenotype data. In order to assess the effectiveness and utility of GEN2PHEN software, we are conducting a series of project evaluations with the clinical and research communities, using the software to explore and interpret areas of biomedical importance. The second evaluation focuses on three areas.

(1) Generating LRGs to describe BRCA1 and BRCA2. At present, many genes have multiple locus specific databases (LSDBs) developed by different research groups. This can create problems for clinical scientists since it may not be clear how variant data in these databases can be reconciled. GEN2PHEN has developed the LRG (Locus Reference Genomic) standard to describe reference sequences and is developing web services to allow LSDB data to be accessed by other programmes.

(2) Development of a BioMart for LSDB data. This allows users to compose complex queries over multiple datasets, such as genetics variants and phenotype ontologies.

(3) Routine calculation of the consequence of genetic variation. Genome resquencing projects are generating large quantities of variation data. This creates a need for software that enables the consequence of genetic variation to be determined. The SNP Effect Predictor annotates variants using the Ensembl API and databases.

For each area we will present tools to researchers and clinicians and gather feedback. This will be used to ensure that deliverables remain relevant to needs.

P11.052 Advances in gene expression microarray profiling from small amounts of adenocarcinoma and normal total RNA samples

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Gene expression profiling by microarray analysis provides an important avenue for understanding biological mechanisms, classifying tissue and tumor types, and identifying signs for diagnosis and prognosis. To address the need for high sensitivity gene expression profiling of low quantities of total RNA, we have developed a modified linear amplification procedure that generates quantities of Cy-labeled cRNA suitable for oligonucleotide microarray experiments from total RNA input amounts as low as 10 nanograms. This new procedure, available in the new Low Input Quick Amp Labeling Kit, employs the AffinityScript Reverse Transcriptase, a mutant MMLV-RT that binds primer-template complexes with 10-fold higher efficiency than wild type MMLV-RT, resulting in increased cDNA yields and improved sensitivity from smaller sample inputs. The protocol uses a single round of IVT amplification without purification of the cDNA product resulting in labeled cRNA in less than one day, enabling gene expression profile comparisons in less than two days. Comparisons of probe signals from technical replicate samples demonstrate high reproducibility with wide dynamic ranges, and generally comparable signals across a broad range of input amounts. This new labeling approach was used to detect differences in gene expression between cancerous and normal cells using new gene expression arrays with updated content resulting in gene expression profiling results consistent with the current literature.

P11.053 Next generation sequencing to reveal new variants into cancer research

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The human genome lies at the core of human disease research. The remarkable technological advances in sequencing have enabled the sequencing of individual human genomes.

Using next generation sequencing technology such as Illumina/Solexa, we could get an all-round view of complex diseases including cancer at DNA level, RNA level, and epigenetic level.

Applying whole genome resequencing and with a high enough level of coverage, somatic mutations from cancer genome can be obtained. The accompanying pathogen genomes will also be collected.

Exome capture sequencing is a relatively economical strategy at the present stage, which is more suitable. This study will analyze the exome of several human cancers by concurrently sequencing several cancer exomes and their paired non-disease tissue. Cancer-specific genetic mutations will be identified by directly comparing exon sequences from a patient's tumor to its non-disease exons. In addition, SNP genotypes will be calculated for each patient as well as structural variations.

Next generation sequencing could also be applied to RNA of cancers to investigate the first phenotypic effects of all the DNA changes. Starting with RNA, using transcriptome sequencing, digital gene expression profiling and small RNA sequencing technologies, cancer-specific alterations in expression levels, aberrant splicing and fusion transcripts could easily be defined.

With further adaptation sequencing technology could be extended to detect epigenetic changes of the cancer genome. Whole-genome bisulfite sequencing, MeDIP-sequencing and ChIP-sequencing are used to discover aberrant DNA methylation and histone modifications, which are now well established in the development and progression of cancer and other complex diseases.

P11.054 Display of high-throughput genomic data in the UCSC Genome Browser

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The UCSC Genome Browser has become an valuable tool for the analysis of genomic data. Attracting over 3 million hits a week from more than 80,000 different IP addresses per month, it provides access to genome annotations, links to other online resources and an integrated view of disparate datasets.

Increasingly focused on data of relevance to human health, the browser has released data tracks displaying annotations from the Database of Genetic Association Studies of Complex Diseases and Disorders (GAD), Online Mendelian Inheritance in Man (OMIM), Genome-wide association studies from NHGRI (GWAS), the Database of Genomic Variants (DGV), commercial gene-chip probe mappings for comparative genome hybridization and other datasets valuable to research and clinical geneticists..

These public data are displayed together, mapped against the UCSC Genes track and data from dbSNP, and the ENCODE, HapMap and other projects, providing users with a graphical view of complex data from many sources at once. Users can upload their own lab data to view alongside these resident tracks.

With the increasing availability of next-generation sequencing, many laboratories are generating large datasets requiring genomic coordinate mapping. New data formats are now available for the UCSC browser to create a custom track from private data on the browser without uploading gigabytes of data. We describe how to use the compression and indexing scheme that creates a file on the user's machine. This is accessed by the Genome Browser, which extracts only the information needed for the custom track at the display coordinates .

P11.055 Comprehensive in silico prediction of mRNA splicing effects in BRCA1 and BRCA2 variants

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The influence of variants of uncertain significance in the BRCA1 and BRCA2 genes has confounded testing for predisposition to and heritability in breast cancer. Variants are often singletons, in which the phase is unknown, and the familial history is either sparse or unavailable. Using information theory-based analysis, we examined the all variants in the Breast Cancer Information Resource for potential effects on natural splice sites. BRCA1: Among 505 missense changes, 5 are splicing mutations that abolish 2 donor sites and 15 are leaky splicing mutations that occur at 4 acceptor and 5 donor sites. Most leaky mutations have <20% of normal splice site strength, however some have modest effects. Of 58 synonymous codon substitutions, one abolishes and 3 are predicted leaky donor site splice sites. Of 124 intronic vari-

ants, 13 presumably abolish splicing and 38 are predicted to be leaky; intronic cryptic splice site activation is predicted for 10 acceptor and 7 donor sites. BRCA2: Of 916 missense changes, 2 abolish a single donor splice site, 15 are leaky splicing mutations (at 8 donor sites) and 5 activate in frame cryptic splice sites. One of 63 synonymous changes abolish splicing and 5 reduce splice site recognition. Of 161 intronic changes at natural sites, 35 are predicted to be leaky and one activates a cryptic acceptor splice site. Splicing mutations in BRCA1/BRCA2 that coincide with intronic, silent and/or missense changes affecting the same splice site are frequently leaky, suggesting that high levels of these gene products are essential for tumour suppression.

P11.056 Mutation Screening by High Resolution Melt Analysis in early onset forms of Hereditary Spastic Paraplegias.

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The hereditary spastic paraplegias (HSPs) are an etiologically heterogeneous group of neurological disorders which results from the selective degeneration of upper motor neurons, of which key diagnostic clinical findings are spasticity and pyramidal weakness of lower limbs. Although genetically diverse with 46 genetic loci for HSP and 20 genes identified, it is often difficult to separate the disorders on clinical grounds.

This study aims at genetic-molecular investigation of families affected by early onset forms of ADHSP, ARHSP, and apparently sporadic cases, based on HRM, direct sequencing and MLPA techniques.

We performed HRM technique (LightCycler 480, Roche Molecular Systems) to screen two "early onset" HSP genes, *SPG3A* and *SPG5A*. We studied a total serie of 9 unrelated autosomal dominant and 5 autosomal recessive hereditary spastic paraplegia families, and in 18 apparently sporadic patients, manifesting either pure or complex forms of the disease. Then by direct sequencing we analyze samples with putative mutations.

Sequencing the *SPG3A* gene disclosed a novel heterozygous missense mutation in exon 12, observed also in another family showing similarity in phenotype. On the whole we identified six mutations, of which three novel: one segregating in two unrelated ADHSP families, two in two unrelated ARHSP families, the others in apparently sporadic cases.

Our data confirm an high mutational frequency in *SPG3A* and *SPG5A* genes in early onset forms of ADHSP and ARHSP (respectively 20% and 7%) and apparently sporadic cases.

P11.057** Molecular Strategies for Hypertrophic Cardiomyopathy Genetic Analysis: correlation between genotyping and expression data

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Hypertrophic Cardiomyopathy (HCM) is the most common hereditary cardiovascular disease. The benefits of a gene-based diagnosis for both basic research and clinical medicine are limited by the high considerable costs of current genetic testing strategy due to the elevated number of genes and mutations involved in HCM. We demonstrate coupling Mass Spectrometry Genotyping and High Resolution Melting as a new strategy for HCM diagnosis. This strategy allowed us to identify HCM known mutations and several new mutations in sarcomere and cytoskeleton genes including a mutation in *CSRP3* gene, not usually studied in current HCM genetic diagnosis. In a new approach we hypothesized that sarcomere gene transcripts represent molecular markers for HCM evaluation being gene expression profile an indicator

of the cardiac remodeling process. Real-Time analysis of sarcomere genes have been done using RNA extracted from interventricular septum and skeletal biopsies from HCM patients. Unsupervised machine learning methods were used to distinguish differences between groups of patients, tissues and genes. Clustering algorithms revealed two main groups both with a strong correlation between the genomic expression pattern in cardiac and skeletal muscles for MYH7 and TNNI3 genes. The transcriptional differences between the two group clusters could be due to different morphologic HCM features and to DNA genetic profile. Moreover, the correlation (sarcomere-skeletal) transcriptional profile will allow the inference of skeletal muscle as a valid biomarker of HCM. Together the new genotyping strategy and the establishment of a correlation of the genetic-transcriptional profile will have important implications for HCM clinical management and prognosis.

P11.058 Induced pluripotent stem (iPS) cells for disease modeling of neurodevelopmental disorders

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Congenital neurological and neurodevelopmental disorders constitute a heterogeneous group which affects 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. The recent progress in genomic reprogramming of human somatic cells into induced pluripotent stem cells (iPSC) followed by lineage specific induction has provided a tool for the study of human disorders. With the availability of patient derived somatic cells, e.g. fibroblasts, the method offers a unique opportunity to study and model specific human disorders that affect neuronal cells.

We have established somatic cells from different neurodevelopmental disorders including lissencephaly, leukodystrophy, agenesis of corpus callosum and acrania. These cells are being reprogrammed to iPSC and analyzed for pluripotency, i.e. potential to differentiate into cells of the three germ layers, and differentiated into neuronal cells. iPSC derived neuronal subtypes will be scored for number, morphology, migration and apoptosis. iPSC from normal individuals are used as controls. The expected results from this effort will provide novel and basic understanding about pathophysiological mechanisms in neurodevelopmental disorders.

P11.059 Transgenerational methylation changes induced by the endocrine disruptor vinclozolin on imprinted genes

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Endocrine-Disruptors have been suggested to affect the reproductive system. In this study, Vinclozolin, an endocrine-disruptor with anti-androgenic properties, was administered at low doses (50mg/kg/day) to pregnant mice at the time of embryo sex determination. The possible effects on the differentially methylated domains of 5 imprinted genes: H19, Gtl2, Peg1, Snrpn and Peg3 were examined in the male offspring, by bisulfite treatment - pyrosequencing, over 3 generations. Both somatic and germ cells were analysed. In the sperm of controls the percentages of methylated CpGs were close to the expected theoretical values. However, in the *in utero* exposed mice, VCZ induced impressive changes in the percentages of methylated CpGs of all 5 genes. The effects of VCZ were transgenerational, with however a gradual disappearance from F1 to F3. No uro-genital malformations were noticed. A possible effect on spermatogenesis was explored. Interestingly, the mean sperm concentration of the VCZ-administered female offspring was severely decreased as compared to the controls, in the F1 generation. The effect was no more significant in the F2 and F3 generations.

These drastic changes in the pattern of methylation of the examined imprinted genes, in the germ cells of the offspring, congruent with a deleterious effect on sperm concentration, suggest that the observed methylation changes may indeed be responsible for the observed spermatogenic impairment.

P11.060 Evaluation of cellular activity status by *in situ* Proximity Ligation Assays

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Although there are several methods for determining levels of proteins in tissues and cells, there is a great need for more selective methods as well as methods that will enable detection of the functional state of proteins. The activation status of proteins is in most cases regulated by post-translational modifications (PTMs) such as phosphorylation that cause structural changes in the proteins and thereby expose catalytic sites or promote interactions with other proteins. By binding partner proteins, i.e. forming protein complexes, they will gain additional function such as the ability to target substrate molecules. There is also a need to measure many parameters in single cells, such as protein levels, protein activity, mRNA expression and DNA sequence to obtain a more accurate view of the status of the cell and to identify cell-to-cell variation in a heterogeneous cell population such as a tissue section. The activity status of a protein or signaling pathway can be visualized with *in situ* Proximity Ligation Assays using a pair of antibodies targeting the interacting proteins or PTM, using an attached DNA molecule to the antibodies to template the creation of a circular DNA molecule that is a surrogate marker for the interaction. It can then be amplified by rolling circle amplification and detected with a single-molecule resolution in fixed cells or tissues. Combining this method with padlock probes, for detection of DNA and mRNA with a single-nucleotide resolution, now gives us the tool to monitor cellular activity status in clinical material.

P11.061 Optimization of the protocol for DNA purification from blood for Thermo Scientific KingFisher Flex

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Thermo Fisher Scientific, Vantaa, Finland.*

The pure and intact DNA or RNA is an important starting point for various experiments. Thermo Scientific KingFisher Flex offers an efficient and rapid automated system for isolation of high quality nucleic acids. The technology is based on magnetic rods transferring particles through several purification phases and it is adaptable for all magnetic particle separation kits.

To obtain the optimal yield and ratio of purified DNA we analyzed the mixing speeds of the protocol for DNA isolation from blood. Faster mixing speeds were found to increase the yield of DNA and additionally the slow mixing speeds resulted in the impurities in the elution buffer. However, especially in the heated steps with simultaneous mixing the slow speeds are necessary.

In several applications the highest possible ratio of isolated DNA is not the prime goal but instead the briefness of the protocol is essential. We compared conventional and rapid protocols for DNA purification from different volumes of blood. The results indicated that although DNA isolated with the conventional protocol had better quality and quantity than DNA isolated with the rapid protocol, the latter proved to have sufficient quality for several downstream applications, for example for PCR.

The KingFisher® Flex together with the easily modified Thermo Scientific BindIt software provides large variety of options to create different purification protocols. Mixing combinations can be optimized depending on the starting material, the purified product or demands on the rapidity of the protocol.

P11.062 An automated analysis protocol for research of KRAS/BRAF mutation detection for data generated on capillary electrophoresis instruments

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Biomarker research continues to be an important focus in oncology studies, including the role of KRAS and BRAF mutations in CRC and other EGFR-associated cancers. This has led to increased research of these genes as possible predictive markers and targets for continued study.

With this increased interest comes a need for automation of data analysis and report generation to decrease bottlenecks in the research laboratory by reducing manual review time. This poster will present an

automated workflow for detection of KRAS and BRAF mutations and concise report generation in sample data generated on capillary electrophoresis instruments using fragment analysis software tools. We will demonstrate how key features in the software, such as sample quality values, allele binning and report analysis, enable this workflow to be a significant improvement over visual scoring methods.

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

P11.063 KRAS mutation screening by HRM analysis: a comparative study

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Background: Colorectal cancer (CRC) is one of the most common human malignancies with more than 300.000 cases both in the United States and in the European Union each year. Kirsten RAS (KRAS) is a small GTPase that plays a key role in Ras/mitogen-activated protein kinase signaling pathway (GO:0008284); somatic mutations in the KRAS gene are frequently found in many cancers and result in a constitutively active protein. Furthermore the responses to anti-EGFR monoclonal antibodies therapies in colorectal cancer are associated with the KRAS mutational status

This study aims at performing the KRAS mutation detection on formalin-fixed paraffin-embedded (FFPE) tumor tissues by High Resolution Melting analysis (HRM) and compare the results with sequencing analysis.

Methods: Thirty FFPE samples were macrodissected to enrich samples for tumor cells. KRAS gene amplification was performed using two different sets of primers for HRM and direct automated sequencing.

Results: Our results showed a strong concordance between the HRM analysis and cycle sequencing (29/30, 96,6%); however the HRM could not predict the type of mutations. In only one sample (3.3%) the HRM analysis showed a mutated profile which was not subsequently confirmed by direct sequencing.

Conclusion: We plan to expand our cases to validate its efficacy as a prescreening tests, also in terms of specificity and sensitivity.

Our preliminary data support the notion that HRM analysis could be an affordable test and valuable methodology for the detection and screening of somatic KRAS mutations in clinical samples prior confirmation cycle sequencing.

P11.064 Detection of translocation breakpoints in a leukemia cell line by SOLiD(TM) sequencing of mate paired fragments

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The aim of this project is to combine current massive sequencing technology and bioinformatics to generate a sequence-based karyotype with kilobase resolution. As a pilot project we have performed a translocation analysis on a whole genome sequence generated from a B-cell leukemia cell line previously characterized using G-bandning, M-FISH and affymetrix SNP.6 array. Two mate-paired libraries were constructed using two different fragment sizes. The sequenced libraries contained the 25 bp paired-ends of fragments with an average size of 1,5kb and 3,2 kb. A total of 16*109 basepairs were obtained of which 5,5*109 were present in paired fragments. The translocation analysis was performed by selecting reads matching to two different chromosomes and sorting them according to the position of the first and then the second tag. The fragments were clustered in pairs according to the library fragment size. Groups with at least four fragments and present in both sequencing runs were retained while those present in an independent control sample were removed. This filtering resulted in 50 groups of fragments representing hypothetical translocation breakpoints, including two of the translocations characterized cytogenetically. Breakpoint analysis revealed the juxtaposition of an enhancer element located on 12p to the MYC gene on 8q, as the underlying change of one of the previously known translocations. Experimental verification of the remaining hypothetical rearrangements will be performed. In summary sequencing of mate paired fragments can easily identify interchromosomal rearrangements at kilobase precision but identification of significant rearrangements relevant to disease patho-

genesis still remains a bioinformatic and experimental challenge.

P11.065 COMPARING HAPLOTYPES IN THE PRESENCE OF NOISE: APPLICATION FOR LINKAGE STUDIES

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The availability of high-throughput data enables detailed comparison of genomes between individuals. This holds great promise for genetic mapping, especially in isolated populations, where Identity By Descent (IBD) haplotypes are found in loci associated with autosomal recessive diseases. Implementing a simple framework for pair-wise IBD inference, we address the two main challenges in IBD mapping: unreliable estimates of allele frequency and marker errors. In this study we propose a Hidden Markov Model (HMM) for comparing haplotypes between pairs of individuals and demonstrate its efficacy in whole genome scans for linkage analysis. A comparison of haplotypes is mapped to a new marker space, {0, 1}, which represents the IBS (Identical by State) status for each marker between the individuals. The distribution of the new marker is less sensitive to allele frequencies and allows a direct assessment of mistyped markers in the candidate IBD regions.

P11.066 Blood transcriptome of childhood malaria

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We recently identified apoptosis as an important factor in generating lymphopenia during malaria. In this work, we pursue a deeper understanding on the genetic mechanisms underlying this disease process. To this end, we investigated the genomic transcriptional profiles of whole blood of healthy children and children with asymptomatic infection, uncomplicated malaria, malaria associated with severe anemia and cerebral malaria. We hybridized 5 U133A and 5 U133B Affymetrix microarrays for each condition. The profiles were confirmed by FACS (CD35, CD55 and CD71) and real-time PCR (24 genes) results and compared with GEO data from other authors. We also used Ingenuity systems software to find immune pathways significantly affected by gene regulation. A set of immunoglobulin gene segments was repressed in asymptomatic infection, as well as genes involved in B cell receptor signaling and dendritic cell maturation. Genes and corresponding pathways become activated in uncomplicated malaria, as well as TLR signaling. Genes involved in lymphocyte ICOS-ICO-SL signaling, IFN-signaling, HLA class II antigen presentation and in the cross-talk between dendritic and NK cells were repressed during severe malaria. Allergenic eosinophil and basophil genes were also repressed. Severe malarial anemia was specifically characterized by reticulocytosis and altered expression of complement regulatory proteins, whereas only a few genes encoding inflammatory products were activated during cerebral malaria. The transcriptional profiles found in blood cells of children with severe malaria suggest an imbalance of key regulatory processes, the restoration of which by novel supportive therapies could prevent adverse outcomes.

P11.067 Analysis of association of the polymorphic locus Fnu4HI in the gene for monoamine oxidase MAOA with indicators of nonverbal intelligence of human.

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Monoamine oxidase A (MAOA) - an enzyme that plays an important role in the metabolism of neuroactive and vasoactive amines in the central nervous system and peripheral tissues. The gene encoding MAOA, localized on the X chromosome (p11.4-p11.3). The analysis associations of polymorphic variants Fnu4HI the gene MAOA with the level of nonverbal intelligence of human.

Materials and methods. Analysis Fnu4HI polymorphism in the gene MAOA conducted in 200 unrelated individuals aged 18-35 by polymerase chain reaction (PCR) with subsequent processing amplifikons

appropriate restriction endonuclease. The level of intellectual development (IQ) in the subjects was determined by the method of Cattell. In accordance with the index IQ study population were divided into two groups: high (above 110 points) and normal (90-110 points) level of intellectual development.

Results. Analysis of the association evidence of significant differences in the distribution of genotype frequencies between the study groups due to increased frequency of heterozygous genotypes MAOA + / - (36.84% vs 19.75%, P = 0.002) and reducing the frequency of genotype MAOA - / - (48.42% vs 65.43%, P = 0.035) in the group of individuals with high levels of intellectual development.

Thus, the association established polymorphic locus Fnu4HI in the gene MAOA with indicators of nonverbal intelligence.

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P11.068 Gene expression changes after losartan treatment in patients with Marfan syndrome

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Marfan syndrome (MFS) is a common genetic disorders with an incidence of 2-3 in 10 000 live births. Aortic dilatation is the main cause of morbidity and mortality of Marfan patients. Losartan, an antihypertensive drug inhibits the aortic dilatation in a mouse model of MFS.

Methods

We analyzed gene expression in snap frozen punch skin-biopsies of 26 patients before and after 4 weeks of losartan therapy using Human Exon 1.0 ST Array (Affymetrix). Whole Transcriptome Gene Expression (WTGE) analysis in 52 samples was analyzed using Significance Analysis of Microarrays (SAM) method for paired samples. Separate analyses of pathways involved in MFS and alternative splicing under losartan were performed. Results were validated using rtPCR.

Results

WTGE analysis revealed 79 differentially expressed genes ($\Delta=0.54$, $q<5\%$). When analyzed for functional annotation using Fatigo, lipid metabolic process was the most significant term ($p=7.1 \times 10^{-10}$). From the WTGE and pathway analysis 7 most significant genes with highest fold change were validated using rtPCR (Table 1). Alternative splicing analysis revealed 2 significant genes, ACSM3 and ADCY6 ($\Delta=0.3$, $q=0$), known to be involved in left ventricular hypertrophy, blood pressure regulation and obesity.

Conclusion

Clinical dosage of losartan seems to decrease TGF- β signaling pathway by modulating the expression in CIDEA and ENG genes in Marfan patients. The exact role of genes involved in lipid metabolic process in MFS is yet to be defined.

| Genes with prominent expression levels change under losartan therapy | | |
|--|-------------|---|
| Gene ID | Fold Change | Function |
| NRNPH2 | 1.22 | Nuclear mRNA splicing |
| CPS1 | 2.22 | Proteolysis, novel association with homocysteine levels |
| GAL | 2.05 | Insulin secretion, adaptive responses to acute orthostatic stress preventing syncope in susceptible individuals |
| CIDEA | 1.26 | Negative regulation of TGF β pathway |
| ENG | 0.85 | Regulation of TGF β signaling pathway, modulation of immune response |
| FADS2 | 1.81 | Unsaturated fatty acid biosynthetic process, metabolic syndrome |
| FADS1 | 1.59 | Unsaturated fatty acid biosynthetic process, metabolic syndrome |

P11.069 K562 cells stably expressing hnRNP C1/C2 shRNAs have growth inhibition

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MBD2 acts as a repressor on the human γ -globin gene. However the protein does not act by binding directly to CpG residues in the γ -globin promoter. In one model, MBD2 may act by binding indirectly to γ -globin regulatory regions through an associating complex. One such complex identified in K562 cells is the LCR-associated remodeling complex (LARC). Here we have set out to test this model. Previous results from our lab suggest that MBD2 siRNAs can augment γ -globin expression

in K562 cells. However siRNAs directed at the DNA binding protein of LARC (i.e. hnRNP C1/C2) do not have the same effect. To further these findings, we have embarked on creating K562 cells stably expressing hnRNP C1/C2 shRNAs using lentiviruses. To this end, five hnRNP C1/C2 shRNA-pLKO.1 vectors were screened in HEK 293T cells. Two gave $\geq 60\%$ target transcript knockdown levels after normalization for transfection efficiency. These vectors were subsequently packaged and used to transduce K562 cells. Cells transduced with these vectors grow very poorly relative to those infected with the pLKO.1 control vector, suggesting that hnRNP C1/C2 down-regulation causes growth inhibition. We are in the process of determining the hnRNP C1/C2 and γ -globin expression levels of these cells using qRT-PCR.

P11.070** Relevance of the novel MeCP2 target genes SCG10 and PN1 in the pathogenesis of Rett syndrome

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Rett syndrome (RTT) is a progressive neurodevelopmental disorder that occurs almost exclusively in girls, with an incidence between 1/10,000 and 1/15,000 births. Classical RTT is characterized by a period of apparently normal development until 6-18 months of age, followed by a period of regression, with deceleration of head growth, loss of speech and acquired motor skills. More than 90% typical RTT patients have heterozygous mutations in the X-linked MECP2 gene that encodes the methyl-CpG-binding protein 2, a transcriptional modulator. In a previous study, we identified SCG10 and PN1 as potential novel MeCP2 target genes in human primary fibroblasts. Here, the significant SCG10 down-regulation was confirmed by quantitative RT-PCR and western-blot in the Mecp2^{308Y} mouse model, either in adult cerebral tissues or in a primary cortical neuron culture. Down-regulation of PN1 was also confirmed by shRNA experiments targeting MECP2 in the human neuroblastoma-derived SH-SY5Y cell line. SCG10 is a neuron-specific protein that accumulates in the central domain of the axonal growth cone where it acts as a potent microtubule-destabilizing factor enhancing neurite outgrowth, while Protease-nexin 1 (PN1) is a protease inhibitor secreted by the astrocytes, with postulated roles in regeneration and regulation of neurite outgrowth. In the adult mammalian nervous system PN1 has been shown to be expressed in the olfactory system and by some glial cells in response to neuronal injury. Deficit in both proteins may explain in part how MeCP2 deficiency affect neuronal function, leading to reduced dendritic branching and short dendritic spines observed in the RTT cortex.

P11.071 MeCP2_270 mutant protein is expressed in astrocytes as well as in neurons and localizes into the nucleus

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MECP2 gene encoding for methyl-CpG-binding protein 2 (MeCP2) is a transcription factor that recognize methylated CpG dinucleotides and binds symmetrically to such target DNA sequence. Mutations in MECP2 gene is the main cause for Rett syndrome (RTT). RTT is a progressive neurodevelopmental disorder, which affects primarily girls during early childhood and it is one of the most common causes of mental retardation in females. The R270X is one of the most frequent recurrent MECP2 mutations among RTT cohorts. The R270X mutation resides within the TRD-NLS (Transcription Repression Domain-Nuclear Localization Signal) region of MeCP2 and causes a more severe clinical phenotype with increased mortality as compared to other mutations. To evaluate the functional role of R270X mutation, we generated a transgenic mouse model expressing MeCP2₂₇₀-EGFP (human mutation equivalent) by BAC recombineering. The expression pattern of MeCP2₂₇₀-EGFP was similar to that of endogenous MeCP2. Strikingly, MeCP2₂₇₀-EGFP localizes in the nucleus, contrary to the conjecture that the R270X could cause disruption of the NLS. Quantitative expression analysis of MeCP2 target genes revealed up-regula-

tion in Mecp2_270_EGFP transgenic mice as compared to wild type mice. In primary hippocampal cells, we show that MeCP2_270_EGFP was expressed in astrocytes by co-localization with the astrocyte specific marker, glial fibrillary acidic protein. Our data showing expression of MeCP2_270_EGFP in the transgenic mice astrocytes further reinforce the recent findings concerning the expression of MeCP2 in the glial cells.

P11.072 A New High-Speed Conversion Kit for the Direct Detection of Methylated DNA Extracted from gDNA, Cancer, Blood or Cell Line Tissues for Research Utilizing Capillary Electrophoresis

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DNA methylation influences cellular differentiation and higher organism development. DNA methylation in the promoter region of genes plays a central role in epigenetics and is associated with transcriptional silencing. Controlling or regulating gene expression by DNA methylation is related to: cell cycle regulation, embryonic development, X-chromosome gene silencing, chromatin structure and Oncogenesis.

A methylation kit has been developed to provide a faster, more streamlined research protocol that allows direct bisulfite conversion from biological samples (cell lines, tissues, blood and purified DNA) with comparable or better performance than existing kits. Without pretreatment of DNA with bisulfite, methylation patterns are lost after PCR amplification of DNA. Traditionally, the bisulfite conversion incubation step has been time consuming and labor intensive. Treatment of DNA with bisulfite reagent converts C-bases to uracil (U) without significant conversion of the 5-methyl cytosine (5mC), subsequently resulting in PCR amplification of 5mC as C-bases and the U-bases are read as thymine (T-bases) on capillary electrophoresis platforms. Available bisulfite conversion methods require DNA extraction from paraffin, while this procedure is capable of direct bisulfite conversion from unprocessed FFPE specimens. Complete conversion is achieved within 2-3-hrs. We describe a direct bisulfite conversion of gDNA samples within 2-3-hours that has also been tested on cancer, blood, and FFPE samples. These research samples are run in parallel with an existing bisulfite kit and the results compared on the 3500 capillary electrophoresis platform.

The 3500 series Genetic Analyzer is for research use only. Not intended for any animal or human therapeutic or diagnostic use.

P11.073 The role of methylation status of 5' UTR end of ROR2 gene in osteoblast differentiation of MSCs.

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Several transcription factors such as Runx2 and Osterix are involved in osteoblast differentiation. ROR2 is an orphan receptor tyrosine kinase that acts as a co-receptor in Non-canonical Wnt signaling pathway. It is shown that ROR2 expression increases during differentiation of MSCs to osteoblast and then decreases as cells progress toward osteocytes.

MSCs isolation and expansion: human bone marrow mesenchymal stem cells(hBMSCs) is isolated using combining Ficoll-mediated discontinuous density gradient centrifugation with plastic adherence. isolated mononuclear cell layers were then suspended in DMEM and plated in culture flasks and incubated for overnight and then the non-adherent cells were washed away leaving behind the adherent cell population. Flow cytometric analysis was used to identify the isolated hBMSCs.

Osteoblast differentiation: for osteoblastic differentiation, the hBMSCs were incubated for 21 days by Bone Differentiation Medium(BDM) . Alizarin red staining and RT-PCR for ALP and osteocalcin confirm osteoblastic differentiation.

Methylation specific PCR(MSP): MSP is used for analyzing methylation status of 5' UTR end of ROR2 gene in MSCs and osteoblastic cells in different stages. Results:

5' UTR end of ROR2 gene in MSCs is hyper methylated and methylation is reduced as cells differentiate toward osteoblastic progenitors and finally this region is hypo methylated in mature osteoblasts.

Discussion:

This finding confirms previous studies, which showed that ROR2 ex-

pression during osteoblastogenesis is increased as cells differentiate to osteoblasts. ROR2 as a non-canonical Wnt signaling regulates osteoblastogenesis.

P11.074 Quantitative and qualitative analysis of SNRPN gene methylation for Prader-Willi syndrome by real-time PCR with methylation-sensitive high-resolution melting analysis

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Introduction Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are caused by deletion at a common region of chromosomal 15q11-13 or uniparental disomy of chromosome 15 (UPD15). Identification of CpG methylation status at the SNRPN gene locus can be used as a diagnostic test for PWS.

Methods Here we show that real-time PCR with methylation-sensitive melting analysis (MS-HRM) is a sensitive and specific assay detecting CpG methylation status as well as copy number aberrations in a single tube. We established this duplex assay for the analysis of 38 individuals with Prader-Willi syndrome, 2 individuals with Angelman syndrome, and 28 unaffected individuals.

Results By comparing the copy number, deletion type and non-deletion type resulting in real-time PCR with marked copy number changes. In MS-HRM, the peak at lower melting temperature (85 °C) belonged to the paternal allele (unmethylated) while the peak at higher melting temperature (89 °C) belonged to the maternal allele (methylated). The Tm of the two alleles demonstrates clearer distinction in a derivative plot. The genotyping results obtained were fully concordant with traditional methylation PCR and all 68 samples were identified successfully.

Conclusions Our results show that the real-time PCR with MS-HRM strategy is good alternative for molecular diagnosis of PWS and AS. The in-house protocol provides detailed information about deletion and UPD genotypes as a significant strategy in clinical applications for epigenetics in a routine laboratory.

P11.075 High-throughput genomic and tissue microarray expression profiling reveal the placental gene expression signature of early-onset HELLP syndrome similar to that of early-onset preeclampsia

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Background: The involvement of the placenta in the pathogenesis of preeclampsia and HELLP syndrome is unquestionable. In this study we aimed to examine placental gene expression and reveal candidate biomarkers.

Methods: Placental specimens were obtained from women with early-onset preeclampsia and HELLP syndrome as well as from control women who delivered preterm or at term. After histopathological examination, fresh-frozen placental specimens were used for microarray expression profiling followed by pathway analyses. A linear model was used to fit -DCt values as a function of study groups while adjusting for gestational age. Data was further analyzed by qRT-PCR and by immunostainings on tissue microarrays constructed from paraffin-embedded placental specimen.

Results: Placental gene expression was gestational age-dependent among preterm and term controls. Out of the 350 differentially expressed genes in preeclampsia and 555 genes in HELLP syndrome, a large group of 226 genes had expression changes. Of these 226 genes, many encode proteins that have already been implicated as putative biomarkers for preeclampsia. Enrichment analyses revealed that differentially expressed genes are mostly attributed to the same biological processes, expressed in similar cellular compartments and participate in the same pathways in early-onset preeclampsia and HELLP syndrome; however, there were some biological processes overrepresented in only one of these syndromes.

Conclusion: High-throughput genomic and tissue microarray expres-

sion profiling revealed that the placental gene expression signature in early-onset preeclampsia and HELLP syndrome largely overlaps. Accordingly, a common cause and pathophysiological processes may lead to shared placental and clinical features in these severe syndromes.

P11.076 Dynamic miRNA profiles in human diseases

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MicroRNAs (miRNAs) have demonstrated their potential as biomarkers in a variety of studies. A particularly promising approach is the determination of miRNA fingerprints in patients from body fluids rather than tissue biopsies.

We measured miRNA profiles in different body fluids (including blood and serum) of about 1,000 patients suffering from different diseases, including cancer (among others lung tumors, pancreatic cancer, prostate cancer) and inflammatory diseases (multiple sclerosis, pancreatitis, COPD). Analyses were based on all known miRNAs as annotated in miRBase version 12.0 or higher. To extract relevant information from this large data set, we implemented a dynamic miRNA database. This database contains the miRNA profile and clinical information for each sample such as the TNM status of a cancer patient. miRNA profiles from different diseases and controls can be easily extracted and analyzed. In addition, the profiles can be used for classifying patients based on the developed biomarker signatures. Besides the comparison of diseases and controls, the flexibility enables numerous relevant diagnostic tasks, for example distinguishing between lung cancer and COPD patients. Since the database content is steadily increasing, more specific questions can be approached, for example the identification of putative differences between metastasizing and non-metastasizing cancers.

Taken together, our database contains the largest collection of complex miRNA profiles from patients' body fluids and will help scientists and clinicians alike to address many questions of different nature.

P11.077** miR-103 and miR-107 are involved in the regulation of CDK5R1/p35 expression.

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CDK5R1 encodes for p35, an activator of CDK5, which is involved in neuronal migration and differentiation during CNS development. A lot of microRNAs (miRNAs) target sites for twenty different miRNAs have been predicted by PicTar in CDK5R1 3'UTR. A Real-Time PCR of 5 selected miRNAs performed in human cell lines showed an inverse correlation between miR-103 and miR-107 levels and p35 expression, suggesting a negative effect of these miRNAs on CDK5R1 expression. We carried out transient transfection of SK-N-BE neuroblastoma cells with miR-107/103 precursors (pre-miR-107/103) and antagonists (anti-miR-107/103). 48h after pre-miR-107 transfection, a significant reduction of p35 levels was observed, by 38% with 50nM and 49% with 100nM of precursor. Otherwise, if anti-miR-107 (50-100nM) was transfected, an increase of 1.4-1.8 folds in p35 levels was observed, compared to the untransfected control. Similarly, the transfection of pre-miR-103 (50-100nM) or anti-miR-103 (50-100nM) caused a reduction (35-40%) or an increase (76-83%) of p35 levels, respectively. Luciferase assays on 3'UTR constructs cotransfected with miR-103/107 showed that 4 out of 9 predicted target sites might be functional. Deletion of the binding sites is in progress: at present deletion of site 1 shows increasing of luciferase activity, while deletion of site 2 produces no effects. Finally, transfection of miR-103 or miR-107 reduces SK-N-BE migration ability by 47 and 45% respectively following *in vitro* scratch assays. The obtained findings indicate that miR-103 and miR-107 regulate CDK5R1/p35 expression and allow us to hypothesize that a miRNA-mediated mechanism might influence CDK5 activity and its pathway.

P11.078 Real-time quantitation of primary transcripts of microRNA genes

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Regulation of mature microRNA (miRNA) expression has been shown during the course of biogenesis under different contexts: from transcription, processing of pri-miRNA to pre-miRNA by Drosha, to processing of pre-miRNA to mature miRNA by Dicer. A mature miRNA can be processed from two or more stem-loop precursor loci, which are denoted with numeric suffixes such as -1, -2, etc. As of the 14th Release of the miRBase, there are 154 genes among the 750 miRNA genes that belong to this category. To interrogate regulation of miRNA expression at the transcriptional level for those that can be processed from multiple genomic locations, it requires assays to specifically quantitate the precise locus of interest. We have developed a pipeline to design and select real-time RT-PCR assays to quantitate primary transcripts of miRNA genes. These assays are designed against genomic DNA sequence so the genomic DNA needs to be removed by prior enzyme treatment. We showed that the TaqMan® Pri-miRNA Assays have minimally detectable or no background using either cell lysates or commercial tissue total RNA. Excellent linearity was seen using cDNA synthesized from total RNA, or using 1ng/ul or greater of genomic DNA. The specificity of the assays to distinguish loci that are processed into identical mature miRNA was demonstrated using subcloned plasmids, as well as using breast cancer cell lines that showed concordant expression patterns among such loci with published data in human breast cancer.

P11.079 MicroRNAs with a potential influence on SERCA2 protein expression in human myocardial infarction

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Cardiac sarco(endo)plasmic reticulum calcium ATPase-2 (SERCA2) plays a central role in myocardial contractility. SERCA2 mRNA and protein reduction may contribute to various form of heart disease. SERCA2 is regulated at post-translational level by phospholamban, and since correlation between decreased SERCA2 mRNA and protein levels is not always observed, we purposed that it may be in addition regulated at post-transcription level. MicroRNAs (miRNAs), post-transcription regulators of gene expression, have been reported to influence a wide variety of physiological as well as pathological conditions, including cardiovascular disease. Since correlation between SERCA2 and miRNA expression was not previously reported, we focused on miRNAs that could potentially influence SERCA2 expression in human myocardial infarction (MI).

SERCA2 protein expression, analyzed by western blot, was decreased in infarcted tissue when compared to remote myocardium, and 21 miRNAs were up-regulated when analyzed by miRNA microarrays in the same tissue. MicroRNA binding prediction to SERCA2 mRNA, using several *in silico* approaches (TargetScan, PicTar, miRBase, miRanda, microrna.org, miRDB, RNA22, mFold) identified 213 putative miRNAs. By combining miRNA microarray, target prediction and western blot results, we identified 8 miRNAs (*miR-34a*, *miR-214*, *let-7i*, *miR-100*, *miR-122*, *miR-199-3p*, *miR-199-5p* and *miR-497*) with a potential influence on SERCA2. In a proportion of samples, up-regulation of *miR-122* was confirmed using real-time PCR, and *miR-100* up-regulation and SERCA2 down-regulation is in accordance with other research performed on cardiomyopathies and neonatal cardiomyocytes. Our results indicate that some miRNAs, in addition to phospholamban, may be involved in the regulation of SERCA2 expression in MI.

P11.080 Real-time quantitation of custom-multiplexed TaqMan® MicroRNA Assays

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MicroRNAs (miRNAs) are ~22 nucleotide endogenous RNA molecules that play an important role in the regulation of developmental and physiological processes in animals and plants. Studies indicate that miRNAs are efficacious biomarkers for the classification of tumors, and are involved in the multilevel regulation of gene expression. Over 10,000 mature miRNAs for hundreds of species are listed in miRBase and a growing number of novel miRNAs are being discovered. To determine the level of miRNA expression, Applied Biosystems de-

veloped the stem-loop RT based TaqMan MicroRNA Assays, a robust method showing large dynamic range, high specificity and sensitivity. We have recently developed a protocol to allow custom-multiplexing of the stem-loop RT primers, for a faster, more flexible approach for miRNA validation. In combination with an optimized pre-amplification method, we evaluated randomly selected miRNA assays in multiplex pools of 12, 24, 48, and 96 primers. Expression correlation for the common assays in the various multiplex was >0.98. Average delta Ct between individual miRNA assays and custom pooled assays was 1.0. Seven 96-plex of randomly selected miRNA assays were also evaluated with total RNA from brain and placenta. Results showed minimal background with only 0.7% of the assays, from a total of 672, with Ct < 35 for the no template reactions. In addition, comparable expression pattern was observed using the delta, delta, delta Ct approach in which >93% was <1.0 when normalized to an endogenous control, and the different methods and samples.

P11.081 RNA expression profiles of Familial Hemiplegic Migraine mouse models with relevance to migraine-associated cerebellar ataxia**

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The CACNA1A gene encodes the $\alpha 1$ subunit of voltage-gated $\text{Ca}_{v}2.1$ Ca^{2+} channels. Mutations in CACNA1A are associated with familial hemiplegic migraine (FHM), a rare monogenic subtype of migraine with aura that can be accompanied by cerebellar ataxia and/or epilepsy. Two extremes of the FHM clinical spectrum are seen with missense mutations R192Q and S218L. Whereas R192Q mutation carriers suffer from pure FHM, S218L patients have a severe phenotype with FHM, cerebellar ataxia, seizures, and brain edema after mild head trauma. Recently, knock-in *Cacna1a* mouse models were generated that carry either the R192Q or S218L mutation. Here we investigated their RNA expression profiles in the occipital cortex and cerebellum because of their relevance to aura and ataxia, respectively. Despite pronounced consequences at molecular and neurobiological level, only modest differences in gene expression were observed in the cortex. This was not due to lack of power in our study since we observed extensive differential expression of genes in the chromosomal region surrounding the CACNA1A gene mutation, which are probably not related to the phenotype of the mice. Expression differences were much more pronounced in the cerebellum of S218L mice and could be linked to their ataxic phenotype. Most strikingly, tyrosine hydroxylase, a marker of delayed cerebellar maturation, is strongly up-regulated in cerebella of S218L mice. In addition, neuronal pathways, such as biogenic amine synthesis pathways show signs of up-regulation as well. Our findings indicate that migraine-associated phenotype cerebellar ataxia in $\text{Ca}_{v}2.1$ mutant mice is best reflected in the basal RNA expression profiles.

P11.082 Accurate distinction of pathogenic from benign CNVs in Mental Retardation

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Copy number variants (CNVs) have recently been recognized as a common form of genomic variation in humans. Hundreds of CNVs can be detected in any individual genome using genomic microarrays or whole genome sequencing technology, but their phenotypic consequences are still poorly understood. Rare CNVs have been reported as a frequent cause of neurological disorders such as mental retardation (MR), schizophrenia and autism, prompting widespread implementation of CNV screening in diagnostics. In previous studies we have shown that, in contrast to CNVs found in the general population, MR-associated CNVs are significantly enriched in genes whose mouse orthologues, when disrupted, result in a nervous system phenotype. We have developed and validated a novel computational method (GeCCO) that differentiates between benign and MR-associated CNVs using genomic features to annotate each CNV. This method is able to achieve a high accuracy when classifying a cohort of individuals with unexplained MR (~1,800). We demonstrate the benefit of applying such computational methods to the interpretation of CNVs,

including those for which the inheritance cannot be determined, as well as rare inherited CNVs. These results indicate that our classification method will be of value for objectively prioritizing CNVs in clinical research and diagnostics.

P11.083 Functional analysis of SNPs affecting mRNA splicing linked to genome-wide association study (GWAS) loci

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We have investigated alternatively spliced mRNA isoforms in genes whose abundance and structure are altered by allele-specific splicing effects. We used information theory-based models of constitutive splicing to search for splicing mutations among all validated HapMap Phase II SNPs. We developed software (calculating ΔR_i in bits) to predict mRNA splicing effects associated these polymorphisms and annotate genomic sequences with information changes at natural and potential cryptic splice sites. 3097 SNPs produced $\Delta R_i \geq 1$ bit ($\geq \Delta 2$ fold) splice site changes in natural splice sites (963 donors, 2134 acceptors). To support these predictions, exon microarray expression data from HapMap Phase II individuals (GEO Accession GSE7851) was used to determine which SNP-affected natural sites show allele-specific changes in splicing index (the ratio of the exon to core probe-set cDNA hybridization intensities). Public mRNA and EST libraries were also queried for supporting evidence of cryptic site activation, as well as diminished use of affected natural sites. Predictions were confirmed by analysis of the abundance of each predicted splice isoform for each SNP genotype. Using real time RT-PCR, 11 out of 12 (and one equivocal) SNPs tested with $\Delta R_i > 1$ bit exhibited significant changes in splicing consistent with levels predicted by information theory. Genome-wide, strengths of 16906 cryptic and natural splice sites ($\Delta R_i > 0.9$ bits) were found within 10kb of SNPs significantly associated with various phenotypes by GWAS. The most common phenotypes included 2799 SNPs proximate to GWAS SNPs associated with CNS-related disease, 2004 with cardiac diseases, and 2110 with obesity-related diseases.

P11.084 Arrayed Primer Extension (APEX-2) as a flexible multiplex PCR-based method for nucleic acid variation analysis

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Mutation detection and SNP genotyping are basic techniques in human genetic- and molecular diagnostic laboratories. Currently numerous commercial and custom assays are available, but none of them is prioritized due to diverse applications and therefore "one size doesn't fit for all". The key parameters for successful genotyping methods are accuracy, flexibility, high throughput, and inexpensive cost. We developed an up to 640-plex multiplex-PCR protocol and combined it with four-color arrayed primer extension (APEX) detection method, APEX-2. In APEX-2, each studied locus is amplified in multiplex PCR and detected on a microarray. For multiplex PCR, two oligonucleotides are required for each locus. Primers in the multiplex reaction are mixed together and involved in the first phase of PCR, using a standard protocol. All formed products are then amplified in the second, universal primer phase of PCR. After purification and concentration of the multiplex-PCR products, single base extension on an oligomicroarray is performed and genotypes are called.

The principle of APEX-2 was published in 2008 and after that, many applications using various plex sizes and different DNA sources simultaneously (mitochondrial, autosomal, and Y chromosome DNA) have been carried out. Furthermore, we have studied DNA extraction methods from blood and saliva for multiplex PCR, scanned optimal primer design parameters and looked deeply into all reaction and cycling conditions. APEX-2, as a custom genotyping assay has high accuracy (99.86%), flexibility (50 - 1,000 plex) and throughput, and has a potential to become a reliable and user-friendly genotyping tool.

P11.085 Molecular neurocytogenetic, *in silico* and proteome (interactome/reactome) evaluations of brain-specific somatic genome variations in neurodegenerative diseases

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Chromosome instability (CIN) is associated with neurodegeneration in Alzheimer's disease (AD) and ataxia-telangiectasia (AT) (Iourov et al., 2009). Here, we have attempted to determine cellular and molecular basis of this relationship. High-resolution interphase molecular cytogenetic and *in silico* analyses showed that CIN involves specific chromosomal loci in the AT cerebellum: 14q12 (FOXP1B, NOVA1), 7p14, Xp22.1, Xp22.3 (FRA7C, FRAXB, FRAXC). The AD brain demonstrated a dramatic 10-fold increase in levels of chromosome 21-aneuploidy (6-15%). We further performed a proteomic (interactomic/reactomic) study analyzing either loci disrupted by CIN or crucial intracellular processes in context of our data. Genes disrupted in the AT cerebellum interact with SMAD pathway (SMAD2, SMAD4 at 18q21.1) of TGF-beta response, required for proper apoptosis and cell cycle progression. Additionally, these data implicate that neuronal-specific alternative splicing is likely to be affected in AT (NOVA1 disruption). Reactomic study suggested that G1/S DNA damage checkpoint p53-dependent pathway is altered by ATM mutations only without additional CIN effect. According to our and literature data, it is supposed that G2/M DNA replication checkpoint pathway should be primarily affected in the AD brain. However, neither interactome nor reactome evaluation has elucidated elements playing a role in this pathway that have been found to be involved in AD brain-specific CIN. Therefore, we concluded that neurodegeneration in the AD brain is mediated by DNA-replication-independent aneuploidization and chromosome specificity is achieved by natural selection during brain ontogeny. We conclude that bioinformatic and proteomic analysis is a required addition to studies discovering mechanisms of complex multisystem disease.

P11.086 Resequencing of the candidate region for 16q-ADCA and detection of an insertion polymorphism by fragment assembly data using massively parallel short-read sequencing.

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Next Generation Sequencing technologies have brought new approaches of genetic analyses for personal genome or human diseases. The technologies can accurate giga-base order of sequence within a single run, allowing for complete resequencing of whole candidate region of a genetic disease in patients, whose responsible gene is not identified.

We picked one candidate region of a neurogenetic disorder, the autosomal dominant spinocerebellar ataxia linked to chromosome 16q22 (16q ADCA), as an example of the resequencing approach. The candidate region of 16q ADCA spanning 500kb was extracted and amplified in Japanese individuals by long-range PCR. Then, a fragment library from the region was constructed and sequenced to a depth >400-fold using the SOLiD 3 system. Comparison of each sequences and the genome database allowed us to find novel variations in Japanese individuals in addition to known SNPs.

Recently, an insertion polymorphism near the BEAN gene has been found. One type of insertion might be associated with 16q-ADCA.

We investigated whether the fragment analysis but not mate-paired analysis could detect the insertion. A survey for the depth of reads from fragment assembly could detect such insertion.

The resequencing approach of whole candidate region for a genetic disease might be a powerful tool to find not only SNPs but also in/del mutations in patients.

P11.087 Next generation sequencing: setting a new stage for DNA diagnostics?

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DNA diagnostic care suffers from laborious, time consuming procedures and limited possibilities to screen all genes for a given disease. This results in incomplete, expensive diagnostic care and long reporting times. Here, Next Generation Sequencing (NGS) provides a unique solution, enabling mutation analysis of all known disease-associated genes in one single experiment. Application of NGS would significantly improve the diagnostic care for both patients, by maximizing their chances on a timely identified causal mutation, and family-members at risk, by enabling carriership-testing in many more cases.

To assess the applicability of NGS in routine diagnostics, we compared the performance and quality of NGS using an Illumina-GAI to currently used methods.

Mutation detection was performed in 10 patients diagnosed with Arrhythmogenic Right Ventricular Cardiomyopathy. For these patients the ARVC-associated genes DSC2, DSG2, PKP2, DSP, JUP, DES, PLEC1, and TMEM43 were analyzed. These eight genes were PCR-amplified and subsequently analysed by NGS and CSCE/Sanger sequencing.

Our results indicate that NGS allows the readily detection of all mutations when using in-house developed software tools and appropriate parameters for data analysis. The variations detected included 2 pathogenic mutations and 12 uncertain variants. There was a 100% concordance between the two methods. NGS could indeed provide a mutation detection method that performs better than currently used techniques. A considerable reduction of reporting times is feasible using NGS for the mutation analyses of disease genes. Further aspects dealing with data-quality, data-interpretation, detection of false-positive and false-negative results and implications for further implementation will be discussed.

P11.088

Targeted Next Generation Sequencing Pipeline For Large-scale Genomic Studies

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Massively parallel DNA sequencing technologies, combined with sequence capture methodologies, have accelerated the investigation of specific genomic regions while reducing the time, cost, and effort needed to interrogate multiple loci. Combining the SOLiD 3plus System with febit's HybSelect Sequence Capture Solution in one streamlined targeted NGS pipeline, we established a powerful facility to resequence large genomic regions in hundreds of clinical samples per study in a very economical way and within short time.

Demonstrating the power of this targeted re-sequencing pipeline, we present the results of a series of research studies that investigated clinically relevant genomic regions with an overall performance of resequencing several hundred thousand bases in over 400 clinical samples within 4 weeks. Multiplexed analysis of barcoded and pooled samples drastically enhanced throughput and reduced cost per sample in these studies, whose results will be further discussed in upcoming high-impact publications.

The presented facility will allow targeted sequencing of more than 1,000 barcoded samples per week, serving large scale genomic studies and clinical research, thus taking next generation sequencing to the next level.

P11.089 Evaluation of the ABI SOLiD sequencing technology for routine genetic diagnosis: a pilot study on BRCA1 and BRCA2

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designed a pilot study to determine if the SOLiD technology could fulfill the requirements of molecular diagnosis.

Twenty patients fully genotyped by reference methods and harboring various mutations on *BRCA1* and *BRCA2* (transitions, transversions, insertions and deletions from 1 to 20 bp) were included. DNAs were enriched for *BRCA* exonic regions and flanking introns by in-house multiplex PCRs and the Multiplicon assay.

A targeted re-sequencing experiment was performed using the SOLiD v3 system and the molecular barcoding. Four among the 20 barcodes performed poorly but all patients were correctly identified.

By using *BFAST*, 60% of the 100Mb reads were uniquely mapped to the amplicon sequences with an average depth-of-coverage of 3500X. We called potential SNPs and small indels with *SAMTools* and filtered out most of false positives using the minimum coverage, the minimum allele ratio and distribution of the heterozygosity frequency as main filtering parameters. All specific point mutations were successively localized and identified along with expected polymorphisms.

Few variations were found that were absent using our reference method, thus needing Sanger sequencing to decide between SOLiD false positives or diagnostic false negatives. Next steps are the detection of large rearrangements and inclusion of a larger number of genes and patients.

Overall this pilot study shows that the SOLiD system plus a dedicated bioinformatics pipeline fulfill the diagnostic requirements.

P11.090 Multiplex target enrichment using DNA indexing for ultra-high throughput SNP and CNV detection.

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Screening large numbers of target regions in multiple DNA samples for sequence and structural variation is an important application of next generation sequencing but an efficient method to enrich the samples in parallel has yet to be reported. We describe a method that combines DNA samples using indexes or barcodes prior to target enrichment to facilitate this type of experiment. Sequencing libraries for multiple individual DNA samples, each incorporating a unique 6bp index, are combined in equal quantities, enriched using a single in-solution target enrichment assay and sequenced in a single reaction. Sequence reads are parsed based on the index, thus allowing sequence data to be analyzed at the level of the individual DNA sample. We show that use of indexed samples does not impact on the efficiency of the enrichment assay. Using sequencing libraries containing 3- and 9-indexed HapMap DNA samples, the method was found to be highly accurate for SNP identification. Even with sequence coverage as low as 8x, 98.8% of sequence SNP calls were concordant with known genotypes. Additionally, we observed a strong correlation between sequence coverage and copy number variant (CNV) genotype in the indexed samples at a known CNV region ($\rho=1$, $p<0.0005$). This method introduces significant flexibility into next generation sequencing study designs. Within a single sequencing experiment, it has the capacity to analyze the exonic regions of hundreds of genes in tens of samples for sequence and structural variation using as little as 1 μ g of input DNA per sample.

P11.091 Transcriptional hallmarks of Noonan syndrome in peripheral blood mononuclear cells

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Noonan syndrome (NS) is an autosomal dominant syndrome characterized by a distinctive facial appearance, heart defects and skeletal abnormalities, rarely associated with mental retardation or juvenile myelomonocytic leukemia (JMLL). Germline mutations responsible for NS affect genes of the RAS-MAPK pathway, which regulates cell proliferation, differentiation and senescence by ultimately controlling gene expression. To investigate the transcriptional consequences of such mutations, we performed Global mRNA Expression Profiling (GEP)

on Peripheral Blood Mononuclear Cells (PBMCs), a target tissue of the syndrome. We analyzed 28 samples from molecularly defined NS patients (17 with *PTPN11*, 6 with *SOS1* and 5 with the recently identified *SHOC2* mutation), and 21 samples from age- and sex-matched controls. We then selected genes differentially expressed between control samples and each of the three mutation subgroups. Interestingly, the transcriptional signatures so obtained displayed remarkable gene-specificity: each mutational subgroup (*PTPN11*, *SOS1*, *SHOC2*) was well distinguished from control samples by its own signature and displayed limited transcriptional overlap with the other subgroups, which is not consistent with a homogeneous generic NS molecular profile. Systematic leave-one-out cross-validation confirmed that the transcriptional signatures are potentially useful not only to discriminate control from NS samples, but also to subdivide NS samples based on the mutated gene. In silico data mining revealed common features of PBMCs from NS patients, such as functional alteration of the JAK/STAT and γ -Interferon pathways. These data provide evidence of a high potential for GEP analysis of PBMCs both as a diagnostic tool and to dissect the molecular complexity of Noonan syndrome.

P11.092 Improved structural characterization of chromosomal breakpoints using high resolution custom array-CGH

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Array-CGH is a powerful tool for the rapid detection of genomic imbalances. By customizing the array it is possible to increase the resolution in a targeted genomic region of interest and determine the structure of the breakpoints with high accuracy, as well as to detect very small imbalances. We have used targeted custom arrays to zoom in on 38 chromosomal breakpoints from 12 different patients carrying both balanced and unbalanced rearrangements.

We show that it is possible to characterize unbalanced breakpoints within 17 to 20 000 bp, depending on the structure of the genome. All of the deletion and duplication breakpoints were further refined and potential underlying molecular mechanisms of formation is discussed. In one of seven carriers of apparently balanced reciprocal translocations we detected a small deletion of 200 bp within the previously FISH-defined breakpoint, and in another patient, a large deletion of 11 Mb was identified on a chromosome not involved in the translocation.

Targeted custom oligonucleotide arrays makes it possible to perform fine mapping of breakpoints with a resolution within the breakpoint region much higher compared to commercially available array platforms. In addition, identification of small deletions or duplications in apparently balanced rearrangements may contribute to the identification of new disease causing genes.

P11.093 The Opitz BBB/G syndrome protein complex regulates the translation efficiency of the ephrin B1 mRNA

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The MID1 protein, mutations in which cause a hypertelorism / hypoplasia syndrome called Opitz BBB/G syndrome, assembles a ribonucleoprotein (mRNP) complex that associates with mRNAs. The identity of these mRNAs and the potential implications of their interaction with the MID1 protein complex remain unclear. We show here that the ephrin B1 (EFNB1) mRNA associates with the MID1 protein complex. Ephrins are pathfinder molecules regulating embryonic patterning. Mutations in EFNB1 have been identified in patients with craniofrontonasal dysplasia, another hypertelorism syndrome. We furthermore show that the MID1 protein complex regulates the translation of EFNB1, which is remarkably reduced in cells from OS patients leading to a compensatory increase of EFNB1 transcription and an impaired responsiveness towards ephrin receptor stimuli in these cells. Our data not only bring further insight into the pathogenesis of OS and other hypertelorism syndromes, but also provide evidence for a novel and unexpected mechanism regulating EFNB1 function.

P11.094 Involvement of SOX9 and FGF23 in RUNX2 regulation in osteoarthritic chondrocytes**T. Orfanidou¹, A. Tsezou²;**¹University of Thessaly, Medical School, Laboratory of Cytogenetics and Medical Genetics, Larissa, Greece, LARISSA, Greece, ²University of Thessaly, Medical School, Laboratory of Cytogenetics and Medical Genetics, Larissa, Greece, University of Thessaly, Medical School, Department of Biology, Larissa, Greece, Larissa, Greece.

Osteoarthritis is a metabolic disease, including altered expression pattern of several genes in articular chondrocytes. Chondrocytes' hypertrophy includes matrix remodelling, proliferation and apoptosis, characteristics associated with the progression of osteoarthritis. We investigated possible associations between RUNX2, SOX9 and FGF23 expressions in articular chondrocytes in order to elucidate their contribution in the osteoarthritic cartilage. SOX9, FGF23, RUNX2 and MMP-13 mRNA expressions were evaluated in osteoarthritic and normal chondrocytes by real-time PCR while MMP-13 protein expression by immunofluorescence. RUNX2, FGF23 and SOX9 were downregulated using small interfering RNA technology. The effect of human recombinant FGF23 on SOX9 and RUNX2 expression was tested in normal chondrocytes. We found higher expression of RUNX2 and FGF23 and a decreased expression of SOX9 mRNA in osteoarthritic chondrocytes compared to normal ($p<0.0001$). RUNX2 downregulation resulted in reduced MMP-13 expression in osteoarthritic chondrocytes and inhibition of SOX9 in increased RUNX2 and MMP-13 expression in normal chondrocytes, while inhibition of FGF23 resulted in reduced RUNX2 expression in osteoarthritic chondrocytes ($p<0.0001$). Silencing of RUNX2 or FGF23 did not affect SOX9 mRNA levels in osteoarthritic chondrocytes. Moreover simultaneous downregulation of SOX9 and upregulation of FGF23 in normal chondrocytes resulted in additive upregulation of RUNX2 mRNA expression. Treatment of normal chondrocytes with hrFGF23 resulted in increased RUNX2 mRNA expression, while it had no effect on SOX9 mRNA expression. We demonstrated convincing associations between RUNX2, SOX9 and FGF23 in relation to MMP-13 expression in osteoarthritic chondrocytes, contributing to a better understanding of the abnormal gene expression and cartilage degeneration processes associated with osteoarthritis.

P11.095 The Short Isoform Of P75^{NTR} As A Major Player In Postnatal Hippocampal Signal Transduction**M. A. Sabry, F. Al- Rowaihi, M. Al-Ramadan, A. Bader;**
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The pro-nerve growth factor p75 neurotrophin receptor (P75^{NTR}) is a member of TNF receptor superfamily. Oligoheteromeric signaling of P75^{NTR}, in association with the ligand-selective neurotrophin receptor, tyrosin kinase receptor (Trk) promotes cell survival. P75^{NTR} signalling, in the absence of Trk association, has crucial functions in apoptosis, facilitation of hippocampal long-term depression (LTD) and regulation of axon growth/arborisation and Schwann cell migration.

In a quantitative RT-PCR approach, we studied the expression of the full-length isoform (L) and the short isoform (S) of P75^{NTR} during postnatal development of the rat hippocampus and explored their differential modulation by PTU- induced hypothyroidism initiated during prenatal development.

The most important result that stands out in this investigation is the high postnatal expression level of S isoform of P75^{NTR}, which is comparable to that of the L isoform in control and hypothyroid rat hippocampus, both at ages 2 and 6 weeks, which would reflect important functional postnatal roles of the S form. In control hippocampus, the expression levels of the L isoform is comparable at ages 2 and 6 weeks, while the short isoform is down-regulated, but is still considerably expressed, at age six weeks compared to its expression level at age two weeks. In hypothyroid hippocampus, each of L and S isoforms is significantly down-regulated between ages 2 and 6 weeks. Hypothyroidism significantly down-regulates the hippocampal expression of both L and S isoforms of P75^{NTR}, only at age 2 weeks. The investigation highlights the potential functional significance of L-S partnership in postnatal murine P75^{NTR} signalling.

P11.096 Jörg D. Hoheisel¹, Nathalia Giese², Markus Büchler², Andreas Keller³, Andrea Bauer¹**J. D. Hoheisel¹, N. Giese², M. Büchler², A. Keller³, A. Bauer¹**¹Deutsches Krebsforschungszentrum, Heidelberg, Germany, ²Department of Surgery of the University of Heidelberg, Heidelberg, Germany, ³febit biomed, Heidelberg, Germany.

Our research aims at the development and immediate application of new technologies for an analysis, assessment and description of both the realisation and regulation of cellular function from genetic information.

Pancreatic cancer is at the centre of attention. Global studies are under way on SNP- or mutational variations, the epigenetic modulation of gene promoters, differences in transcription factor binding, changes of transcript levels of coding and non-coding RNAs, on the actual protein expression and the intensity of protein interactions. We also pursue genome-wide knock-out studies for the identification of genes that are critical for tumour development and progression.

By combining the data, we aim at an understanding of cellular regulation and its biological consequences. In combination with clinical facts, the knowledge is used for the creation of means of early diagnosis, accurate prognosis and the analysis of treatment results as well as the establishment of new therapeutic approaches.

As part of the above, we performed a comparison of variations in the abundance of miRs in blood and tissue of patients with pancreatic cancer or chronic pancreatitis and samples from healthy donors. Apart from identifying miRs, which are prognostic markers for survival, we also found miRs that discriminated clearly between tumour, pancreatitis and normal. In addition, we identified a dozen miRs that varied their transcript levels identically in both tumour tissue and blood, while only one would be expected by chance. Their functional roles within tumour cells as well as possible intercellular activities will be discussed.

http://www.dkfz.de/funct_genome/

P11.097 Positional integratatomic approach in identification of genomic candidate regions for Parkinson disease**A. Maver, B. Peterlin;**

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Parkinson disease (PD) is the second most common neurodegenerative disorder, characterized by progressive symptoms of tremor, rigidity, and bradykinesia. Genetic factors have been implicated in its etiology; however precise genetic basis of PD is unclear. Recently, abundance of data originating from studies employing high-throughput technologies to reveal alterations in PD on genomic, transcriptomic, proteomic, and other levels, offer the possibility to integrate this information into a comprehensive picture of changes occurring in PD.

In our study, datasets from various types of studies in PD (linkage, genome-wide association, transcriptomic studies of brain and blood samples and proteomic studies) were obtained from online repositories or were extracted from available research papers. Subsequently, human genome assembly was subdivided into regions, and signals from aforementioned studies were sorted into corresponding regions according to their position in the genome. For each study type, regions were then prioritized according to signal significance and number of signals located in each region. Sum of ranks from different studies was used in final prioritization of genomic regions.

We have identified 5 genomic regions containing overlaps of significant signals originating from 5 different study types and 98 regions containing overlaps from 4 different study types. Identified regions contain plausible candidate genes for PD, in addition to genes previously associated with PD.

We present a novel approach for identification of PD candidate genes, based on positional integration of data across various types of omic studies. Furthermore, this approach can easily be extended for identification of candidate genes in other complex diseases.

P11.098 Integrative microRNA and proteomic approaches in Parkinson's Disease (PD)**M. Martins^{1,2}, A. Rosa^{3,2}, N. Charro⁴, B. V. Fonseca^{1,2}, S. Violante², L. C.**¹Instituto de Medicina Molecular, Lisbon, Portugal, ²Instituto Gulbenkian de Ciéncia, Oeiras, Portugal, ³Universidade da Madeira, Madeira, Portugal,⁴Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon, Portugal, ⁵University of

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PD is the second most prevalent neurodegenerative disorder, affecting over one million individuals worldwide. The main pathological hallmark of PD is the loss of dopaminergic neurons within the substantia nigra, leading to insufficient formation and action of dopamine in the basal ganglia circuitry. The cardinal clinical signs are muscle rigidity, resting tremor, bradykinesia and, in more advanced cases, postural instability.

This complex disease is governed by many genetic and non-genetic factors. To identify novel susceptibility genes for idiopathic PD, we are using the "genomic convergence" approach with microRNA and proteomic profiling. This strategy converges data from genetic studies with expression profiling experiments.

We conducted microRNA expression profiles in peripheral blood mononuclear cells of 19 PD patients and 13 controls, using microarrays spotted with probes for 763 human microRNAs. 18 microRNAs were differentially expressed and pathway analysis of their predicted target genes revealed an over-representation of pathways recently linked to non-Mendelian forms of PD. We also carried out a proteomic analysis in pooled blood serum of 30 PD patients and 28 controls using a 2D-DIGE approach. 41 differentially expressed spots were obtained and 23 proteins have already been identified. Isoforms and post-translational modifications are being evaluated and their type and contribution to the disease mechanisms will be further analysed. The microRNAs and respective target genes, as well as proteins differentially expressed will be tested for association with PD. We believe that this approach will allow us to identify specific novel genes/transcripts playing a role in the etiopathogenesis of idiopathic PD.

P11.099 The *Slc26a4* loop mouse mutant, a model for Pendred syndrome, has defects in biominerization, with unique calcium oxalate stone formation in the inner ear

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Human mutations in *SLC26A4* lead to a non-syndromic and syndromic form of deafness, DFNB4 and Pendred syndrome, respectively. Here we show inner ear mineralization defects in a new mouse mutant identified in an N-ethyl-N-nitrosourea (ENU) screen, named loop, that carries a recessive missense mutation in a highly conserved region of *Slc26a4*, rendering this mouse a model for human deafness. Defects were identified in biominerization, the formation of minerals, which is part of the process that produces skeletons, shells, and teeth. Impaired halide transport activity resulted in the formation of giant calcium oxalate mineral bodies not found in the body under normal circumstances and described here in the inner ear for the first time. Infrared and Raman spectroscopy, together with high resolution scanning electron microscopy and immunohistochemistry of otoconia components, demonstrated drastic changes in crystal morphology and composition. Detailed histological analysis revealed that the giant minerals are ectopically distributed within the vestibular apparatus, mimicking imbalance conditions in human as a result of displaced otoconia.

P11.100 Whole genome pharmacogenomic analysis of bipolar disease patients under lithium treatment

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Bipolar Disorder (BD) is a lifelong psychiatric disease characterized by manic and depressive episodes affecting 1-5% of the general population. Among the most effective mood-stabilizing treatments, lithium (Li) represents the mainstay in the therapeutic management of acute-

mania and depression in BD and is still to date, the first choice prophylactic treatment. Besides the high rate of excellent Li responders (~30-40%), a significant fraction of patients present patterns of partial or non response to prophylactic treatment with Li. It has been shown that the variability in Li response is strongly influenced by genetic determinants. A large number of studies have investigated the role of genes in modulating the response to Li reporting contrasting findings. In our study, we have genotyped 50 individuals divided in two groups, according to their degree of Li response. The eleven-point treatment response scale we employed (full response cut-off ≥ 7) allowed us to classify 25 BD patients as non-responders (scored with 0) and 25 as full responders (scored with 8 or higher). These patients were genotyped using the Affymetrix Array 6.0 SNP microarrays (Santa Clara, CA, USA). Data were statistically evaluated using the *Genespring* software using a p-value cutoff of 0.01. We have identified 38 SNPs significantly associated with Li response, with p-values ranging from 1×10^{-5} to 4×10^{-6} . The genomic loci hold genes encoding for elements of G-proteins coupled receptors, LIM-domain binding proteins, sodium channels and GABA receptors. This study enriches the battery of genetic biomarkers that would personalize Li treatment of BD patients.

P11.101 Epigenetics changes for the diagnosis of malignant pleural effusions

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DNA hypermethylation in promoter regions of a gene is recognized as an important epigenetic mechanism of transcriptional silencing of tumor regulatory genes during cancer development. This study could provide valuable information for diagnostic purposes.

We collected 60 patients with pleural effusion with diverse etiology: tuberculosis, para-pneumonia, or cancer.

DNA was extracted with a Qiagen Kit and after bisulfite treatment, a methylation-specific PCR (MSP) was performed. We analyzed the following genes: p16/INK4a, BRCA1 and RAR β .

The study population included 37 men (61.7%), with a mean age of 56.98 ± 21 years. Thirty-three patients (55%) were former or current smokers. Malignant pleural effusion was diagnosed in 28 patients, while benign pleural effusion due to tuberculosis or para-pneumonia was present in 18 and 14 patients, respectively.

The promoter hypermethylation frequencies of benign and malignant pleural effusion, respectively were: p16/INK4a (9.4% vs 17.9%), BRCA1 (46.9% vs 35.7%) and RAR β (12.5% vs 17.9%), therefore no significant differences were detected. Promoter methylation of at least one gene was detected in 64.3% of the patients with malignant pleural effusion, while hypermethylation of at least two genes was observed in 14.3% of them.

Our preliminary results show that the methylation status of the promoter region of p16/INK4a, BRCA1 and RAR β has no diagnostic utility for malignant pleural effusion. The methylated condition of some of the benign pleural effusions could be related, as previously reported, with inflammation and infection, besides certain lifestyle factors such as smoking and high-fat diet, that are related with an increased likelihood of cancer risk.

P11.102 A study of angiotensin converting enzyme gene polymorphism in children with pulmonary hypertension

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Angiotensin converting enzyme (ACE) plays an important role in the pathogenesis of pulmonary hypertension. The ACE gene has been considered a candidate gene for contributing to the development of hypertension and cardiovascular diseases. The ACE gene contains a polymorphism based on the presence (insertion I) or absence (deletion D) within an intron of a 287-bp nonsense DNA domain, resulting in three genotypes (DD and II homozygotes, and DI heterozygotes).

In this study we determined whether the deletion (D)/ insertion (I) polymorphism in the ACE gene may be associated with pulmonary hypertension. We performed a case-control study with 26 patients with pulmonary hypertension and 20 healthy controls. Genomic DNA was extracted from peripheral blood leukocytes by standard methods. The ACE genotypes of the patients were determined through restriction enzyme digestion of a PCR fragment (PCR-RFLP). There were identified 34,61% (9) DD homozygotes, 53,84% (14) DI heterozygotes and 11,53% (3) II homozygotes. No statistical significance differences in allelic distribution were detected between groups.

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P11.103 Novel variation c.1-219G>A in PRKN promoter converts Sp1 binding site to MZF1 binding site

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We have observed five variations in the promoter region of the PRKN gene by direct sequencing. Three of the variations are novel. The influence of the five variations on the binding sites of transcription factors was studied using "TFSEARCH ver.1.3" on the <http://www.cbrc.jp/research/db/TFSEARCH.html>. The analysis showed that variations c.1-324A>G, c.1-65C>T, c.1-42T>C have no significant effects on binding sites of transcription factors, but c.1-355T>G and c.1-219G>A, respectively, affect binding sites of the Cp2 and Sp1 transcription factors. C.1-355T>G causes the deletion of a binding site for Cp2; based on reported data, frequencies of the relevant alleles at this position were not significantly different between PD patient and control groups. The novel variation c.1-219G>A converts a binding site of Sp1 transcription factor to a binding site of MZF1 transcription factor. Sp1 regulates the expression of a large number of genes involved in a variety of processes such as cell growth, apoptosis, differentiation and immune responses. MZF1 is expressed in totipotent hemopoietic cells as well as in myeloid progenitors. Some findings suggest that Mzf1 can act as a tumor/growth suppressor factor. The frequencies of the G and A nucleotides at position c.1-219 in PRKN in PD patient and control groups are not known. The possible relation of c.1-219G>A in the promoter of PRKN needs to be investigated. Genetic variations in promoter sequences have been shown to affect susceptibility to various complex diseases.

P11.104 QKI-7 regulates expression of interferon-related genes in human astrocyte glioma cells

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The human QKI gene, called quaking homolog, KH domain RNA binding (mouse), is a candidate gene for schizophrenia encoding an RNA-binding protein. This gene was recently shown to be essential for myelination in oligodendrocytes. QKI is also expressed in astrocytes, but its function in these cells is not known. We studied the effect of small interference RNA (siRNA)-mediated QKI depletion on global gene expression in human astrocyte glioma cells. The most significant alteration after QKI silencing was the decreased expression of genes involved in interferon (IFN) induction ($P = 6.3 \times 10^{-10}$), including IFIT1, IFIT2, MX1, MX2, G1P2, G1P3, GBP1 and IFIH1. All eight genes were down-regulated after silencing of the splice variant QKI-7, but were not affected by QKI-5 silencing. Interestingly, four of them were up-regulated after treatment with the antipsychotic agent haloperidol that also resulted in increased QKI-7 mRNA levels. These results show that QKI-7 levels and IFN-related gene levels are co-regulated in astrocytes. Furthermore, our findings suggest a novel role for QKI-7 as a regulator of IFN production in astrocytes.

P11.105 Recurrent rearrangements and fixations form the structure of low copy repeat RCCX

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Low copy repeat RCCX in major histocompatibility complex region on human chromosome 6 consists of four duplicated genes, C4, CYP21, STK19 and TNX. One active gene and one pseudogene belong to every duplicated gene pair except for C4, which has two active copies. RCCX is prone to rearrangement that generates additional duplications, conversions and deletions resulting in monomodular, trimodular chromosome structures and the complete lack of active CYP21 (CYP21A2), the most common cause of congenital adrenal hyperplasia. A series of allele-specific long PCR covering the whole region of RCCX has been developed in order to investigate the structure of RCCX. This technique has been enabled to sequence three copies of identical gene from a diploid genome and the haplotypes of CYP21A2 gene in many cases. Unique CYP21A2 haplotypes determined a few specific chromosome variants with two CYP21A2 copies in Caucasians. Phylogenetic analysis was performed on CYP21A2 haplotypes derived from mono-, bi- and trimodular RCCX to trace the derivation of chromosome variant with duplicated CYP21A2. It was shown that recurrent rearrangements generated these chromosome variants, and the newly arisen chromosomes underwent independent fixation processes. The structure of chromosomes variants with duplicated CYP21A2 implies distinct recombination mechanism, and their recurrent generation shed light on some evolutionary aspects of human low copy repeats.

P11.106 Next Generation Sequencing for Extended Applications in Basic and Clinical Research

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Advances in Next Generation Sequencing are rapidly taking place. As a result, a broader range of scientists may apply the technology to their basic or clinical research. Scientists conducting basic or clinical research often design and utilize assays for tagging and counting applications (Whole Transcriptome and Small RNA) or targeted resequencing analysis. In order to perform such assays in these research settings, the sequencing system must include an end-to-end workflow that is accurate, faster, and easier-to-use. We report on improvements to the hardware, software, and chemistry components of a Next Generation Sequencing platform that provide such solutions for sample preparation, sequencing and analysis.

P11.107 Investigation of RNA-seq as a tool for SNP detection.

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The development of Next Generation Sequencing offers the potential of new methods for mapping and quantifying transcriptomes. In particular, RNA sequencing (RNA-seq) has been used for measurement of transcript abundance, studying the diversity of splice isoforms and testing allelic influence on gene expression. Given that the majority of disease related SNPs are likely to be located in coding regions, we investigated RNA-seq as a method of identifying sequence variants (SNPs) in the transcribed regions of the genome. We performed RNA-seq on lymphoblast cell line RNA samples from a trio of HapMap samples that have been whole-genome sequenced. The sequencing method per sample was 40bp single reads in 2 lanes of an Illumina flow cell. Genes were categorised based on their coverage levels and RNA-seq data was compared to the existing 'positive control' DNA sequence data. For genes (n=5077) where sequence coverage was greater than 20X, 92% of SNPs identified in the data were true sequence variants. For the same set of genes, 82% of expected variants were successfully identified. We also detected a number of novel SNPs that Mendelised within the trio, but were not reported from the published whole-genome sequencing data. Whole-genome resequencing is the most comprehensive method of variation detection but it is costly. We demonstrate that in addition to transcriptome analysis, RNA-seq can detect a very high proportion of sequence variation in expressed genes. It is potentially a very useful technique for detecting coding SNPs in disease tissue samples.

P11.108 Key steps in finding pathogenic variants for monogenic diseases using targeted and exome resequencing data**

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With the development of efficient enrichment strategies for next-generation sequencing it is now possible to either screen specific genomic loci (e.g. linkage regions) or even the entire human exome for genomic variations causing disease. We will discuss our approach and highlight key criteria to successfully identify disease-genes from such large data sets. For this we used two datasets, one using the NimbleGen array enrichment approach in combination with Roche 454 Titanium sequencing for analysis of all coding sequence in a 40Mb linkage region in two families with Familial Exudative Vitreoretinopathy (FEVR). In addition, we applied exome sequencing using the ABI SOLiD 3.5 system in patients with Schinzel-Giedion Syndrome. A key criteria for reliably calling sequence variants is sequence coverage. We found that at least 15-fold coverage is required for reliably detecting variants. This criterion was reached for more than 80% of the targeted exome using the Agilent SureSelect enrichment method and identified approximately 20,000 variants per exome. Up to 95% of these variants has previously been reported in SNP databases. Further prioritization of variants is based on functional consequences of variants, evolutionary conservation, and on combining data from multiple patients. This has allowed us to rapidly identify *TSPAN12* as the causative gene for FEVR and *SETBP1* as the causative gene for Schinzel-Giedion Syndrome. These examples demonstrate the possibilities of next-generation sequencing when combined with robust enrichment systems, highlight the importance of control SNP datasets and show that bioinformatic pipelines are crucial to the rapid identification of disease genes.

P11.109 NBS-NGS: an ultra-throughput selective next-generation sequencing method for clinical diagnostics

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Translating the impact of next-generation sequencing (NGS) at the basic research level into molecular diagnostics is a current technical challenge, to enable analysis of specific sets of candidate genes in multiple clinical samples. The coupling of NGS with front-end technology offers a promising solution if multiplexing can be built-in to ramp-up the whole process. To allow assigning of individual samples and pinpointing variant carriers in each sequenced pool, multiplexing using molecular barcodes represents a logic extension of sample pooling schemes. Indeed, we were in advance to tackle this challenge and developed a New Barcoded Selective NGS (NBS-NGS) approach that combines two technology platforms, which both have in-built multiplexing capability and allow accurate SNP detection. Briefly, barcodes are ligated to individual samples during library preparation, pooled, enriched via microfluidic DNA microarrays, and subsequently resequenced using NGS. The ability of retranslating the barcoded targets of individual samples not only reinforces traditional LIMS (Laboratory Information Management Systems) in regulating data production, but also eliminates the need for special experimental and logarithmic designs of overlapping pooling approaches. The performance of NBS-NGS was tested in three different stages and multiplexing levels. The results reflect a compromise of obtaining reasonable key enrichment measures on one hand and ultra-cost reduction on the other. We believe that NBS-NGS opens up new avenues for comprehensive large scale genomic studies, and its inherent flexibility may render routine deep resequencing for diverse clinical settings feasible. The current state of benchmarking of other technology platforms will also be presented at the conference.

P11.110 Seq + PA: Combining Sequencing (Seq) and Individual Peak Analysis (PA) for Quantification of DNA Methylation and Minor Sequence Variation Detection

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Fluorescent DNA sequence traces generated by Sanger sequencing on automated capillary electrophoresis (CE) instruments primarily reveal the composition and exact sequence of nucleotide bases. However, an additional layer of information may be present in the peak height of a given base reflecting a measure of abundance. Here we report a novel method, Seq+PA, that allows the separation of the four dye traces used in BigDye® Terminator 3.1 sequencing chemistry and inclusion of a 5th LIZ® dye-labeled size standard. This enables alignment by size of multiple samples and subsequent data analysis of each individual peak characteristics, such as size, height and area by GeneMapper® software. We have used the Seq+PA method for direct bisulfite PCR sequencing and were able to detect methylated cytosines in CpG pairs to a level of 5%. We have also applied the method for sensitive detection of somatic mutations. To that end, DNAs with a normal or mutant genotype at codon 12 in the KRAS gene were mixed in various ratios and processed for PCR and Seq+PA analysis. The mutant allele was noticeable at a 5% level. Taken together, the method described here has the potential to enable a more quantitative analysis of DNA sequence traces for sensitive detection of minor sequence variations.

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P11.111 Development of SureSelect™ Target Enrichment System Human All Exon Kit and Indexing Kit for the SOLiD™ System

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The identification of genetic variants and mutations associated with human disease requires the development of a robust and cost-effective approach for systematic resequencing of candidate regions in the human genome. Agilent Technologies has further developed the SureSelect Target Enrichment System to allow for the capture of larger genomic regions of interest as well as the multiplexing of numerous samples and sequencing on the SOLiD System. The inherent deep sequencing of the SureSelect Target Enrichment System in combination with the SOLiD System has easily facilitated sufficient read depth and on-target performance for Agilent's Human All Exon Kit, a kit that covers roughly 38 megabases (or 1.22%) of human genomic regions corresponding to the non-overlapping exons from the NCBI Consensus SDS database (CCDS), including > 700 miRNAs from the Sanger v13 database, and > 300 additional human non-coding RNAs. In addition, the high read depth generated by the SOLiD System not only allows for deep coverage of a large target region such as the human exome, but also for multiplexing numerous samples. With the advent of multiplexing, the ability to achieve targeted enrichment on multiple samples on a single quad is maximized, saving researchers time and money without sacrificing performance. In summary, the SureSelect Target Enrichment System paired with the SOLiD System, provides easy to use in-solution protocols that are automatable and cost-effective approaches to analyze discrete genomic regions with unprecedented depth and accuracy.

P11.112 Towards fully streamlined, quality-assured, automated next-generation gene tests

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Using highly parallel pyrosequencing, we developed a *CFTR* genetic test format in which the complete coding region and exon/intron junctions of the *CFTR* gene are sequenced in an amplicon sequencing approach. Since that pyrosequencing may not correctly call homopolymeric nucleotide stretches, we included tagged ARMS primers at such positions, so that the number of nucleotides can also be called through their tag, besides counting the number of nucleotides that are obtained in homopolymeric nucleotide stretches in reads. To increase robustness, 2 amplicons are generated for each region, in which one test result can be used for quality-assurance of the other test result. Quality-assurance of the complete genetic test process and automat-

ic processing was realized by spiking samples with DNA molecules. These molecular bar codes can be processed simultaneously with the DNA fragments under investigation. The test will be quality-assured from the moment that the sample is spiked. The test will be only valid when the correct spiked sample tag is associated with the molecular bar code at the end of process. Not only can sequencing reveal a mutation, it will also identify the sample carrying the mutation. This allows automatic generation of a report, thereby excluding potential errors compared to reports generated by human intervention when test result and patient information are combined in a genetic report.

This format can be transferred to genetic analysis of any gene.

P11.113 Post-Light Sequencing with Semiconductor Chips

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Ion Torrent Systems has developed a DNA sequencing system that directly translates chemical signals into digital information on a semiconductor chip. This approach leverages a trillion dollars of investment from the semiconductor industry taking advantage of existing state-of-the-art chip fabrication technology, and the entire semiconductor design and supply chain. Unprecedented scalability and cost reduction result from decades of Moore's Law advances in semiconductor technologies that are brought to bear within a few years for DNA sequencing.

Ion Torrent sequencing takes place in semiconductor microchips that contain sensors which have been fabricated as individual electronic detectors, allowing one sequence read per sensor. Current configurations have 1.5 million sensors in a 1 cm² chip, with proof of principle to enable densities over 100 million sensors per chip.

The sequencing chemistry itself is remarkably simple. Native nucleotides are incorporated into the growing strand by native DNA polymerase. As a base is incorporated, a direct electrical measurement of the incorporation event is made and the sequence is read out directly into the digital domain. Thus, sequencing is direct, efficient, and massively parallel, requiring no specialized reagents and no optical systems. Using native DNA chemistry with real time detection enables run times to be very short, on the order of an hour with a throughput on the order of 100 Megabases per hour. We will present data and describe metrics from the adenovirus and *E. coli* genomes.

P11.114 Integration of a novel and intuitive user interface for a new next-generation sequencing platform expands usability in basic and clinical research laboratory settings

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The introduction of high-throughput next-generation sequencing systems is revolutionizing the use of sequencing data for a wide range of genetic analyses. Once limited to ultra-high throughput, large genotyping centers, new instrumentation has been developed that is more amenable to basic and clinical laboratories. Combined with the depth and accuracy of large amounts of sequencing data generated, it is clear this technology has tremendous potential for integration in disease and clinical research. To support this potential for research advancement in personalized medicine, it is important for a sequencing instrument's user interface to be intuitive, reliable and easy to use. A new high throughput sequencing system being developed has a newly designed user interface to guide a researcher from the initial setup to real time monitoring, final data validation and export. With a spike-in process control, the instrument will automatically perform a pre-run sample assessment and provide real time performance reports during the sequencing run. The guided workflow interface streamlines sample setup, provides flexibility for a user to disable individual sample or individual tags of a sample to save time and reagents based on experimental needs. The real time reporting at the instrument or on a compatible mobile device provides the user assurance of optimized data quality and quantity. We will show examples of user interfaces that will enable a broad range of laboratories to use high throughput sequencing for applications such as targeted resequencing or RNA-Seq. For research use only. Not intended for any animal or human therapeutic or diagnostic use.

P11.115 Frequency of serotonin transporter promoter gene polymorphism in opium addict people

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A disturbance in brain serotonergic transmission has been repeatedly reported in association with behavioral disorders. The responses to serotonin reuptake inhibitors appear to be impaired in substance use disorders with co-morbid depression, which reflecting genetic polymorphisms involved in the control of serotonergic activity. The frequency of short ("S") allele 5-HTT promoter polymorphism, that appear to influence gene function by reducing transcription efficiency. The association of polymorphism with substance abuse disorder and major depression (MD) was confirmed.

Thirty one opium dependent subjects, males, aged 20-50 years, entered the study, after informed consent. They were consecutive admissions to the treatment program of the public health service for outpatients' addiction treatment. They were not paid for their participation and accepted to enter the study as volunteers. Thirty one normal volunteer were chosen for control, which were matched in age, sex and socioeconomic situation with cases.

The 5-HTT promoter region was amplified by polymerase chain reaction (PCR). 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). Each gel contained one lane of 50 bp ladder to identify the 450 pb fragment designed as L and the 406 bp fragment designated as S.

The frequency of alleles and genotypes was not different in cases and controls. However, the odd ratio was 1.8 and 1.7 respectively. In conclusion, this study did not show strong association between 5-HTT gene and opium addiction.

P11.116 From rare cell phenotype to rare variant genotype: Analyzing heterogeneous samples with single cell resolution using microdroplet technology

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There is a growing need to examine heterogeneous samples (tumor cells, stem cells, antibody producing cells, or the human microbiome) with single cell resolution. Combining single cell isolation methods with sequencing efforts will advance the understanding of tumorigenesis by elucidating "driver" vs. "passenger" mutations in tumor cell populations and circulating tumor cells, and inform the design of "personalized" tumor-specific therapies.

We are developing a novel microdroplet and microfluidic platform that enables a variety of single cell analyses. Uniform picoliter-nanoliter volume aqueous droplets suspended in an immiscible oil stream provide unique opportunities for fluorescence-based analytical techniques, including novel phenotype-based sorting at rates of several hundred cells per second, and false positive rates of 2-5% and false negative rates of <1%. The encapsulated droplet microenvironment also enables unique sorting modalities beyond current fluorescence-activated cell sorting, including selection based on cell secreted molecules, and highly sensitive sorting based on enzyme amplified signals.

We demonstrate the use of microfluidic-generated single cell droplets in workflows that include phenotype-based capture of desired individual cells from a heterogeneous sample coupled directly to single cell genomic characterization. For example, whole genome amplification within the encapsulated single cell microdroplets can be followed by droplet PCR-based targeted amplification of many thousands of selected genomic loci from each cell, for analysis using any Next Generation Sequencing platform.

P11.117 Potentially functional single nucleotide polymorphisms (pfSNP) in the human genome

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With >14,000,000 non-redundant single-nucleotide-polymorphisms (SNPs) that have thus far been deposited in dbSNP, identifying phenotype-causing SNPs poses significant challenges. Here, we integrated >40 different algorithms/resources to identify ~1,000,000 SNPs from dbSNP with potential functional significance (pfSNPs) based on previous published reports, inferred potential functionality from population-genetics approaches, as well as predicted potential functionality. These pfSNPs are reasonably well-distributed across the entire human genome and are enriched in the promoter and genic regions. We have also developed a customizable, well-annotated pfSNP-web-resource that is aimed at facilitating knowledge-discovery and hypothesis-generation through better data-integration and a biologist-friendly web-interface. For all HapMap-genotyped SNPs, information regarding the distance as well as the r^2 -LD of a nearby HapMap-genotyped pfSNP is given. The pfSNP-resource addresses all ambiguity in SNP information, providing complete information about a particular SNP that will facilitate better hypothesis generation. The query interface is not only user-friendly but highly customizable such that different combinations of queries are possible, using Boolean-logic to facilitate the identification of pfSNPs at a user-specified frequency in a specified-population that is expressed in user-specified tissues and/or belong(s) to a user-specified pathway(s) and/or is associated with user-specified disease/drug pathway(s). Furthermore, to facilitate better hypothesis generation, gene/pathway information is also integrated into the query result. Additionally, detailed related information regarding the functional consequences of the pfSNPs are also provided in the query result to facilitate better understanding of the results and aid in the generation of hypotheses. This web-resource will thus be useful to researchers focusing on SNPs/association studies.

P11.118 Allele-specific copy number measurements on CGH microarrays

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Accurate measurements of genomic copy number are important for understanding developmental disorders, oncogenesis and normal genetic variation. In addition to variation in total copy number, there has been increasing interest in assessing the effects of variation in the number of copies of individual alleles, as estimated from the copy numbers of variant alleles at SNP sites.

We have developed an assay on Agilent SurePrint G3 microarrays that allows concurrent measurement of precise total copy number (CN) and allele-specific copy number (AsCN). Depending on the array design, 10 to 30% of probes are allocated to measure SNPs in parallel to total CN on the same array. By digesting with a restriction enzyme that selectively cuts one variant allele, we can measure the copy number of the uncut variant along with the total copy number of the surrounding genomic region. We have measured genotypes at up to 80,000 SNP sites across the human genome, and typically obtain greater than 99% concordance with published genotypes.

We are able to make reliable SNP calls from single probes, by using a genotyped internal reference to correct for labeling and hybridization bias. The normalized response is linear in copy number and highly reproducible. Log ratio distributions are nearly Gaussian, with well-separated distributions corresponding to different copy numbers.

We describe the novel algorithms we use for measuring CN and AsCN and for estimating our confidence in the genotype calls, and show examples of cytogenetic abnormalities associated with known syndromes.

P11.119 A Balanced, Error-correcting Barcoding System for Multiplexed SOLiD™ Sequencing

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A set of 96 barcoded adaptors designed for the SOLiD™ System have been validated for use with paired end libraries. During library preparation, the P2 adaptor is replaced with a multiplex adaptor consisting of three segments: a primer binding site, a barcode decamer and a P2 PCR priming site. The barcode and target DNA are sequenced as two separate reads from the same strand to identify the libraries of origin of multiplexed samples. Libraries may be pooled at any step after

barcodes have been added and then used in a multiplexed emulsion PCR and deposited into a single spot for sequencing.

The barcode design requires only 5bp of sequencing to distinguish 20-plex samples and 10bp for 96-plex samples. The barcodes are colorbalanced at every position in sets of four. Importantly, clear discrimination between barcode samples is achieved by a minimum Hamming distance of 3 colorspace calls.

The barcoded adaptors were validated by SOLiD(TM) sequencing of *E. coli* fragment libraries. Error rates and quality value (QV) scores for the barcode reads were found to be consistent across the final set. Mapping rates and QV scores were consistent for the *E. coli* reads, indicating minimal effects of the barcode decamers on bead templating and sequencing efficiency. Ongoing development studies include integration of the barcoding system with PCR, array and in-solution based methods of target enrichment for enhanced multiplex processing capability. The SOLiD(TM) barcode adaptors will enable the high levels of library multiplexing afforded by the increasing throughput of the SOLiD(TM) System.

P11.120 Paired end sequencing on the Solid platform

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The SOLiD™ platform is a revolutionary sequencing system that utilizes sequential ligation of fluorescently labeled oligonucleotide probes, enabling high fidelity and ultra-high throughput sequencing. Previous sequencing protocols for the SOLiD™ system have only been available in the forward direction (3' to 5'). Sequencing in the reverse direction (5' to 3') is ideal as it enables fragment library paired end sequencing. To this end, novel ligation chemistries were developed to support 5' to 3' read lengths of up to 35 bases. This paired end sequencing technology will be incorporated into the SOLiD V4 platform, increasing effective read length, maximizing throughput per run, and meeting special research interests, such as whole transcriptome studies.

P11.121 Genetic interaction between Sox10 and Zfhx1b during enteric nervous system development

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The involvement of SOX10 and ZEB2 in Waardenburg-Hirschsprung disease (hypopigmentation, deafness, and absence of enteric ganglia) and Mowat-Wilson syndrome (mental retardation, facial dysmorphys and variable congenital malformations including Hirschsprung disease) respectively, highlighted the importance of both transcription factors during enteric nervous system (ENS) development. The expression and function of SOX10 are now well established, but those of ZEB2 remain elusive. Here we describe the expression profile of Zeb2 and its genetic interactions with Sox10 during mouse ENS development. Through phenotype analysis of Sox10;Zeb2 double mutants, we show that a coordinated and balanced interaction between these two genes is required for normal ENS development. Double mutants present with more severe ENS defects due to decreased proliferation of enteric progenitors and increased neuronal differentiation from E11.5 onwards. Thus, joint activity between these two transcription factors is crucial for proper ENS development and our results contribute to the understanding of the molecular basis of ENS defects observed both in mutant mouse models and in patients carrying SOX10 and ZEB2 mutations.

P11.122 Microarrays study of valproic acid treatment effect on gene expression in spinal muscular atrophy derived human fibroblast cultures.

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Spinal muscular atrophy (SMA) in patients is caused by loss of the survival motor neuron (SMN1) gene, in the presence of the SMN2 gene. As a result of point mutation in exon 7 almost all of SMN2 pre-mRNA is spliced improperly and exon 7 is excluded. Valproic acid (VPA) is an inhibitor of histone deacetylases (HDAC) and one of most potent experimentally tested drug in SMA treatment. It is believed that VPA could increase production of functional SMN protein by two possible ways: (1) increasing SMN2 transcription alone, or/and (2) modulation of SMN2 splicing by increasing transcription of splicing factors such as Htra2-β1 and hnRNP-G. In our in vitro experiments on SMA derived fibroblast cultures treated with VPA, we observed no VPA effect on SMN2, Htra2-β1 or hnRNP-G expression or on increasing protein levels as well. It has been published, but not in association with SMA, that VPA exhibit neuroprotective effect by increasing expression of Bcl-2 in CNS. Based on these facts we decided to investigate general effect of VPA treatment on gene expression and also to investigate differences in gene expression between SMA patients and healthy one's. For that purpose we perform 20 separate cDNA microarray analyses. 141 genes show more than 2-fold up-regulation and 51 down-regulation after VPA treatment and 221/124 genes show different gene expression between patients and healthy control. These sets of genes have been further analyzed using Ingenuity Pathway Analysis Software to predict new possible VPA treatment target genes and genes implicated in SMA disease.

P11.123 Investigation of splicing noise in human aged peripheral blood cells

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Splicing in human is regulated by the interaction of more than 300 proteins and 5 snRNAs with hnRNA sequences. Errors in this process emerge as aberrant splice products. To ensure a high splicing fidelity, the aberrant splice products are degraded by RNA surveillance mechanisms as the nonsense-mediated mRNA decay (NMD). Misspliced in frame exons are e.g. not recognised by the RNA surveillance mechanisms and occur in low frequencies in mRNA populations. We suggested that the frequency of these errors (splicing noise) increases with age. To test this, missplicing of 2 in frame and 2 out of frame NF1 exons was investigated by qPCR of wildtype and misspliced mRNA. As described, splicing noise increases in vitro e.g. by cold shock. We found differences in splicing noise between untreated cultured human fibroblast and after treatment with cold shock or puromycin inhibiting NMD. Though, no correlation to the age of the donors or the population doublings of the fibroblasts in the investigated NF1 exons was found. In peripheral blood cells of 11 healthy donors, no correlation of splicing noise to the age (15y - 85y) could be observed, too. However, the splicing noise increased with the length of the upstream intron. Our studies show, that splicing is a process with a high fidelity during ageing in the investigated peripheral blood cells indicating the significance of correct splicing in proliferating cells.

P11.124 Effect of intronic variants in BMP receptor genes involved in hereditary angiopathies. Comparison of splice site prediction softwares and mRNA genetic testing.

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Pulmonary Arterial Hypertension (PAH) and Hereditary Haemorrhagic Telangiectasia (HHT) are two hereditary vascular diseases in which mutations were described in genes involved in TGF-β signalling. Mutations in BMPR2 are present in more than 70% of familial PAH and in 10-30% of patients with idiopathic PAH. About 70-80 % of HHT patients have mutations in ACVRL1 or Endoglin (ENG) genes. An increasing number of detected variants cannot be classified as either disease-associated mutations or neutral variants. These so-called unclassified variants (UVs) include variants that are located in intronic sequences. The purpose of this study was to test a panel of UVs in

vitro for possible splicing aberrations and compared the accuracy of a variety of bioinformatics approaches for predicting the observed splicing aberrations associated with each UV. We performed *in vitro* molecular characterization of RNAs corresponding to 8 intronic variants in BMPR2, ACVRL1 and ENG. In seven cases, a deleterious effect on RNA splicing was seen. Five splice-site prediction softwares (SSPPs) were used to predict whether an effect on RNA splicing was expected for these variants. 70% of the predictions were informative. No false positive prediction was seen. However we observed 5% of false negative predictions. In particular, no splicing anomaly was predicted for the c.625+10G>A variant in ACVRL1 whereas a deletion in the exon5 was detected by mRNA analysis.

For molecular genetics laboratories, bioinformatics tools for prediction of splicing aberrations are useful but need improvement before being used without supporting molecular studies to assess the functional consequences of a variant.

P11.125 Subtle discrepancies of ASF/SF2 ESE sequence motif among human tissues: a computational approach

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The intron removal during the pre-mRNA splicing in higher eukaryotes requires the accurate identification of the two splice sites at the ends of the exons, or exon definition. However, the consensus sequences at the splice sites provide insufficient information to distinguish true splice sites from the large number of the false ones that populate the primary transcripts. Additional information is provided by cis-acting regulatory sequences that serve to enhance or repress splicing, and that may be exonic or intronic in nature: the splicing enhancers and the splicing silencers, respectively. In our study, we tested by computational approach if the exonic splicing enhancer motif binding to the SF2/ASF SR protein is conserved among several groups of human genes. The results showed that the SF2/ASF ESE consensus was conserved between genes within the same chromosome, within different chromosomes and between different levels of muscular cells differentiation. However, this motif displays subtle variations within the consensus sequence between genes expressed in different tissues. These results can emphasize the presence of different translational isoforms of the SF1 gene encoding for the SF2/ASF, or different post-translational protein maturations in different tissues. This tissue discrepancy can also account for the alternative splicing of several genes between tissues.

P11.126 Mosaic uniparental disomies and aneuploidies as large structural variants of the human genome.

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Mosaicism is defined as the co-existence of cells with different genetic composition within an individual, caused by post-zygotic somatic mutation. Although somatic mosaicism for chromosomal rearrangements have been well described in disease, their frequency in normal individuals is poorly defined. We analyzed signal intensity log R ratios and B-allele frequency data from high density Illumina 1M SNP-array applied to blood or buccal DNA samples of 1,991 adult individuals included in the Spanish Bladder Cancer/EPICURO study. We have found mosaic abnormalities in autosomes in 1.7% samples, including

21 segmental uniparental disomies, 8 complete trisomies and 11 large (1.5-37 Mb) copy number variants. Alterations were observed across the different autosomes with recurrent events in chromosomes 9 and 20. The high cellular frequency of most mosaic anomalies detected and their presence in normal adult individuals suggests that this phenomenon is a more common source of genomic variation than previously recognized. Somatic mosaicism represents a new addition to the expanding repertoire of inter- and intra-individual genetic variation, some of which causes somatic human diseases but also modifies penetrance and/or expressivity of inherited disorders and might influence late onset of multifactorial traits. Capturing and classifying all relevant genomic variation features in cells from different tissues, and at different developmental stages, constitutional or acquired, should lead to a better understanding of our complex and evolving human genome and its relation to both diseases and traits.

P11.127 3DM: determining undetermined variants in genetic disorders using protein super-family data**

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3DM, a tool initially designed for protein engineering, is a system that makes use of protein super-family data to predict effects of mutations. This tool can automatically generate super-family databases that contain many different types of protein related information, such as a highly accurate structure based multiple sequence alignment, mutational information automatically scanned from literature, amino acid contact information, solvent accessibility, etc. All data is connected to each other via the multiple sequence alignment making the protein super-family systems powerful tools for prediction of effects of mutations. These systems have been used to predict specific mutations that improved enzyme features such as catalytic activity, enzyme specificity and thermostability. These results have been published in peer review articles.

Recently, the 3DM tool has been applied to predict the effects of undermined variants (UV's) in genetic disorders. For many genetic disorders there is an increasing amount of publications describing phenotype associated mutations in disorder-related genes. 3DM's literature scanner can automatically collect published disease related mutations and stores these in a protein super-family system. To retrieve, visualize, and analyze stored and new mutation data for their (potential) effect we have developed Validator, a web-based tool specifically designed for DNA-diagnostics. Validator uses all data from a 3DM database to link a genetic disorder to UV's.

P11.128 Development of SureSelect™ paired end Sequencing Kit and SureSelect™ Indexing Kit

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The dramatic increase in throughput of sequencing data from next-generation sequencing platforms has enabled scientists to study the genome with unprecedented depth and accuracy. However, the expense of large cohort studies using a whole genome approach is substantial. Agilent's SureSelect Target Enrichment Kit for Paired-End Illumina sequencing analysis allows in-solution selection of a researcher's specific genes of interest with improved sequencing coverage, enhanced ability to assess structural variation, and increased confidence in SNP calls compared to the single-end protocol. Improvements to the Version 2.0 SureSelect Paired-End Kit have made it faster and easier, while increasing performance. The improvements to the Version 2.0 SureSelect Paired-End Kit have also been applied to the SureSelect Indexing Kit. The SureSelect Indexing Kit, in combination with Illumina's Multiplexing Kit, allows researchers to take advantage of targeted enrichment and the increasing capacity of the Illumina GA. The SureSelect Indexing Kit enables up to 12 gDNA libraries to be uniquely "tagged" and then combined on one flow cell lane, with the advantage of enriching for only those specific genes of interest. The ability to combine targeted enrichment and indexing together maximizes the number of samples that can be sequenced at one time, providing optimum time and cost savings without sacrificing performance. In summary, the SureSelect Target Enrichment Paired-End Version 2.0

Kit and the SureSelect Targeted Enrichment Indexing Kit, with the Illumina system, provide in-solution protocols that are automatable and easy to use. This creates a cost-effective approach to analyze specific genomic regions with unprecedented depth and accuracy.

P11.129 The Agilent Technologies' SureSelect™ Platform: Flexible High Performance Target Enrichment System for Next Generation Sequencing Applications

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Next-generation sequencing technology has dramatically increased the ability to sequence DNA in a massively parallel manner. This improvement in throughput is currently enabling scientists to discover rare polymorphisms, structural variants, and novel transcripts at an unprecedented rate. Nevertheless, routine genetic screens in large cohorts of individuals remain cost-prohibitive through whole genome sequencing approaches. To this end, Agilent Technologies has leveraged its ability to efficiently manufacture high fidelity long oligonucleotides to develop the SureSelect platform, a portfolio of sample-preparation products that enables next-generation sequencing users to focus their analysis on particular genomic loci with substantial cost savings. We hereby demonstrate the flexibility and functionality of the SureSelect in-solution method through the targeted sequence analysis of: (i) genomes of multiple species, (ii) subsets of the human genome such as the exome and disease-focused designs, and (iii) custom content ranging in size, complexity, and chromosomal location. We discuss performance with respect to capture efficiency, uniformity, reproducibility of enrichment, and ability to detect SNPs. The high specificity and excellent sequence coverage across multiple designs demonstrates the utility of SureSelect for a wide variety of applications. More importantly, several of the capture libraries are now incorporated into standardized catalog products for ease of ordering, consistency of performance, and reliability of comparison across multiple laboratories. We also introduce an improved desktop version of Agilent's eArray software module that streamlines custom content design for human, model organisms and any other genome available privately or through the UCSC Genome Browser.

P11.130 High coverage targeted resequencing of matched tumor normal samples and FFPE samples using Selector Technology

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With the advent of novel techniques increasing the DNA sequencing throughput by orders of magnitude compared to traditional Sanger sequencing, a need for improved sample preparation techniques that enrich for multiple genomic loci in parallel has emerged. We present a simple and scalable technique for highly specific amplification of a large number of target sequences based on a dramatically improved selector probe protocol. We applied the technique for deep sequencing of all coding exons of 28 genes known to be mutated in cancer in breast cancer cell lines and matched normal and cancer lung cancer tissues, including formalin fixed paraffin embedded tissue. At 20% of the mean amplicon sequencing depth 98% of the targeted exon bps were covered at mean sequencing depth with a 95% on target specificity. Copy number variations could readily be identified and validated using microarray data. Matched fresh frozen and FFPE samples were used to validate analysis of FFPE material and concordance with Hap-Map genotype controls was 99%. The selector technology provides a powerful approach for single tube amplification and high coverage targeted, scalable resequencing without dedicated instrumentation.

P11.131 Diabetes-associated loci are significantly over-represented among genes bound by TCF7L2 in vivo

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Transcription factor 7-like 2 (TCF7L2) has been implicated in type 2 diabetes and cancer. To uncover its downstream targets *in vivo*, we performed chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-seq) in the colorectal carcinoma cell-line, HCT116, where TCF7L2 is abundantly expressed. In three independent chromatin samples we found 1,357 discrete binding sites within 500kb of 1,352 annotated genes i.e. approximately 6% of all RefSeqs. Pathway analysis of this gene list with the software package, Ingenuity, ranked "Diabetes" as the most significant annotation (P -value = 2.62×10^{-9}). We found TCF7L2 binding sites co-localized with seven of the twenty one (33.3%) type 2 diabetes loci detected by genome wide association (GWAS) to date, namely *CDKN2A/B*, *ADAMTS9*, *CDKAL1*, *FTO*, *PPARG*, *TCF7L2* and *WFS1*. Interestingly, we observed four TCF7L2 binding sites within the *TCF7L2* gene itself, with one in the classical promoter region and three within intron 3. The intron 3 observation is highly notable as this is the precise region of *TCF7L2* previously reported to be very strongly associated with type 2 diabetes. These findings suggest that TCF7L2 may be a central node in the regulation of human diabetes-associated genes.

P11.132 A high-resolution anatomical atlas of the transcriptome in the mouse embryo

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During fetal development organs and tissues acquire specialized physiological functions. This process is dependent on an orchestrated, cell type specific expression of genes. We generated the first genome-wide transcriptome atlas by RNA *in situ* hybridization of an entire mammalian organism, the developing mouse at embryonic stage E14.5. This public resource consists of a searchable database of annotated images that can be interactively viewed. We generated anatomy based expression profiles for nearly 16,000 coding genes and over 400 microRNAs. We identified 990 tissue specific genes that are a source of novel tissue-specific markers for 37 different anatomical structures. The quality and the resolution of the data revealed novel molecular domains for several developing structures, such as the telencephalon, and a novel organization for the hypothalamus. The digital transcriptome atlas is a powerful resource to determine co-expression of genes, to identify cell populations and lineages and to identify functional associations between genes relevant to development and disease.

P11.133 High Coverage Gene Expression Profiling on the Applied Biosystems 3500xL Genetic Analyzer

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Whole transcriptome expression profiling is typically performed using hybridization-based microarray methodologies. However, there are a number of limitations to microarray-based approaches such as low sensitivity and specificity, poor dynamic range, and, importantly, microarray expression profiling results are restricted to specific sequence annotations and content. The HiCEP (High Coverage gene Expression Profiling) method was developed to address the above shortcomings in gene expression profiling and provide a sensitive method for detecting a large proportion of transcripts in both known and unknown genes, with low false positive rate. Here we demonstrate the use of the new Applied Biosystems 3500xL Genetic Analyzer for the detection of transcripts unregulated by ionizing radiation (IR). mRNA samples were prepared from mouse embryonic fibroblasts (MEFs) at 0, 3, 6 and 24 hours after IR exposure. The expression of *p21*, *CyclinG1*, *Gadd45a* was assessed to demonstrate the capabilities of the 3500xL system for gene expression analysis. The optional normalization reagent (GeneScan™ 600 LIZ® Size Standard v2.0) and compatible run module enable increased precision and accuracy in relative peak area or height determinations, which are particularly important for the detection of slight expression changes. In addition, flexible GeneMapper® Software v4.1 was used to create reports and calculations to give user-configured tools for reporting HiCEP results. Further, the expres-

sion changes detected by capillary electrophoresis were compared to TaqMan® Gene Expression Assays for the above transcripts. For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

P11.134 Warfarin resistance genotype assessment on patients with acute coronary syndromes and hypertrophic cardiomyopathy

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Warfarin is the most frequently prescribed oral anticoagulant used for the prevention and treatment of thromboembolic problems. However, the drug has severe side effects of bleeding with an estimate of events occurring at a rate of 1.3 to 2.7 per 100 patient-years. The influence of CYP2C9 and VKORC1 genotypes on warfarin dose has been consistently demonstrated in diverse racial/ethnic patient groups in observational studies and randomized clinical trials suggesting that patients of Asian, European and African ancestry require, on average, lower, intermediate and higher dose of warfarin respectively.

In this research study we made use of the TaqMan® Drug Metabolism Genotyping Assays and the ABI PRISM 7900HT Real Time platform to genotype known variants in the CYP2C9*2 and *3, CYP4F2, and VKORC1 genes in 50 patients with Acute Coronary Syndromes (ACS) and 50 patients with genotyped Hypertrophic Cardiomyopathy (HCM). The genotyping workflow has been also tested on the Applied Biosystems Next Generation Real Time Instrument.

In the ACS patients the following polymorphisms were not in the Hardy-Weinberg equilibrium: rs2108622, rs1799853 and rs1057910 ($p < 0.005$) whilst in the HCM patients the following polymorphisms were not in the Hardy-Weinberg equilibrium: rs1799853 and rs1057910 ($p < 0.010$). In both cases the frequency of the "adverse" allele was more represented in the patients' population than in the controls' population. This might due to a selection bias being patients with difficulties in stabilizing an optimal anti-coagulant therapy. Moreover, the results on the ABI PRISM 7900HT were perfectly reproducible on the Next generation Real Time PCR platform.

P11.135 Whole genome and transcriptome amplification in large biobanks

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Biobanks are a key resource in unravelling the molecular basis of diseases, identification of new targets for therapy and improvement of attribution in drug discovery and development. The scientific trend in biobanking shows the need for stable techniques for amplification of biomaterials, which can be used for samples stored under very different conditions. The focus of the project is the standardisation and validation of the innovative techniques of whole genome amplification (WGA) and whole transcriptome amplification (WTA) in the context of biobanks. A general standardized protocol for WGA and WTA procedures that use Phi29-DNA-polymerase in biobanking will be developed. The major aims of our project are:

1. To establish standardized WGA protocols for large biobanks
2. To develop standardized WGA tools to recover genomic DNA, which is in plasma or serum samples and from FFPE- tissue or blood spots
3. To optimize the WGA procedure by extensive quality control measures of WGA products
4. To develop and establish WTA of large biobank samples
5. To optimize WTA procedures by extensive quality control of WTA products

Furthermore, the concept of the project is to transfer the results of WGA and WTA solution to national and international organisations in the field of biobanking. The development of the proposed, innovative and specialized tools and customized solutions will help to expand and secure biobanks.

P11.136 Characterizing Human Diversity by Whole Human Genome Sequencing of Southern African Genomes

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By using a combination of next generation sequencing technologies and exome enrichment, we have sequenced two complete and named individual genomes from South Africa. These individuals are !Gubi, a Kalaharian Bushmen, and Archbishop Desmond Tutu, representing an admixture comprised of two of the largest African Bantu groups. By focusing on genomes from the southern African, we help establish the extent of diversity within the human genome. For example, we are able to show that the diversity between two bushmen that live less than 1500 km from each other is greater than the diversity observed between a Caucasian compared to an Asian. By using a combination of sequencing technologies, we are able to generate a high quality reference genome that can be used as a comparative tool for the broader community. Initial analysis of !Gubi's genome identified at least 1.3 million SNP's that were novel when comparing to the public reference databases. We used high density genetic arrays to both validate these SNP's and determine what percent are shared verse private. As all participants in this project are believed to be near 80 years in age and in good health, we believe that their genomes can provide a tool to identify alleles that provide protection against disease and in some instances demonstrate that previously indentified disease causing alleles appear to not cause disease in this population. These findings will ultimately help the community understand rare variants across populations that may contribute to disease and potential drug treatment.

P11.137 Ancient DNA research without PCR: the power of true Single Molecule Sequencing.

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DNA extracts from archaeological samples tend to contain low quantities of DNA that is highly fragmented and chemically damaged. In addition the samples are highly contaminated with microbial DNA after being embedded in soil for hundreds or thousands of years. Analysis of this DNA using shotgun sequencing approaches therefore usually yields only low amounts of DNA of interest (a few percent). We reasoned that a major effect was the methodology used, enriching the higher quality contaminating DNA. We tested this hypothesis by analysing five ancient DNA samples of human origin using Helicos' true Single Molecule Sequencing technology (tSMS). The samples were from skeletal remains of five individuals buried between 1225 and 1850 AD in the city of Eindhoven (The Netherlands). The samples were also analysed using Sanger (mtDNA HVR1 and autosomal STRs) and Roche/454 shotgun sequencing. While the methods involving DNA amplification yielded only <5% human sequences, singe molecule sequencing gave yields of up to 40% and more. tSMS sequencing allowed us to determine the gender of all samples and for three the entire mtDNA was covered (average coverage of 30x, some regions >100x). Based on these initial results we foresee a whole range of new studies that can be performed using low quality DNA samples, incl. archaeological, forensic, and archived (FFPE) samples. For our study on the ancient remains, we now suddenly have a range of new possibilities, incl. full genome sequencing, we could not have envisaged before.

P11.138 Exploring the role of miR-590 in Williams Beuren syndrome**

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Williams Beuren syndrome (WBS) is a recurrent genomic disorder caused by hemizygosity of 28 contiguous genes at 7q11.23. Patients with WBS have a characteristic constellation of medical and cognitive findings. Despite a significant advance in the last years still much has to be learnt in order to further translate the knowledge into a better care of patients.

We investigated the role of miR-590, the only miRNA located within

the WBS deleted region. Preliminary results showed that both miR-590-5p and miR-590-3p miRNA strands are downregulated in cell lines of WBS patients. Because individual miRNAs regulate the expression of multiple target genes, we speculated that the downexpression of miR-590 in WBS has strong influences on the generation of the large spectrum of phenotypes because of the wide altered control of its target genes. This project moved from the idea that if we can modulate the action of such miRNA, we will find a possible "large spectrum" therapeutic target for WBS.

In order to asses the biological pathways controlled by miR-590, and to identify the set of its putative target (PTs) genes, we exploited global transcriptome analysis in cells following overexpression and inactivation of miR-590.

Preliminary, by qPCR, luciferase assays, and western blot analysis, we have validated a set of predicted miR-590 PTs identified by querying HOCTAR a recently developed software for miRNA target prediction. We are now performing functional assays on some selected PTs to assess their biological relevance on WBS. The results of these comprehensive analyses on miR-590 will be presented.

P11.139 Using transcription modules to identify pathways perturbed in Williams-Beuren syndrome

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No global assessment of the dysregulations caused by the Williams-Beuren Syndrome (WBS) deletion exists to date. We profiled the transcriptomes of skin fibroblast cell lines from WBS patients and compared them to matched controls. We identified 366 differentially expressed genes that were significantly enriched in extracellular matrix genes, major histocompatibility complex (MHC) genes, as well as genes the products of which localise to the actin cytoskeleton, microsome and vesicular fractions. We then used public expression datasets from human fibroblasts and the Iterative Signature Algorithm (ISA) to establish "transcription modules", i.e. subsets of genes that exhibit a coherent expression profile over a subset of microarray experiments. The majority of these modules are highly enriched in genes with common functional annotation, but they have the added advantage of including genes that have no or fragmented annotation. Hence, this modular approach increases the power to identify pathways dysregulated in WBS patients, thus providing additional candidates for genes and their interactions modulating the WBS phenotypes. Dysregulated modules are often interconnected and share multiple common genes, suggesting that intricate regulatory networks controlled by a few key regulators are disturbed in WBS. Our results emphasize the role of the extracellular space in the pathophysiology of WBS and provide novel clues about other affected processes. We identified, for example, variations in the expression of GABA receptor components that could have consequences at the cellular and synaptic level and common features with other syndromes, such as dysregulation of the Di George Syndrome critical region 2 gene (*DGCR2*).

P11.140 An epigenetic profile of active and inactive X chromosomes correlates gene methylation with inactivation status**

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X chromosome inactivation is a dosage compensation process that silences most genes on one X chromosome in each female cell. In order to characterise epigenetic changes that accompany this process, we generated comparative DNA methylation profiles in both Turner syndrome (45,X), and normal females (46,XX). Methylated DNA was immunoprecipitated and hybridized to tiling oligonucleotide arrays, generating epigenetic profiles of active and inactive X chromosomes. We observed that X-inactivation is accompanied by increased DNA methylation of the inactive X specifically at CpG islands. While both intra- and inter-genic CpG islands are influenced, the biggest increase

in methylation occurs at the promoters of genes silenced by X-inactivation. In contrast, genes escaping X-inactivation have low methylation levels that are similar between active and inactive X chromosomes, while genes that undergo unstable/polymorphic X-inactivation show intermediate increases in methylation proportionate to their frequency of inactivation. Thus promoter methylation and susceptibility to X-inactivation are directly correlated. We utilised this fact to predict nine additional Refseq genes that escape X-inactivation, and performed comparative sequence analysis of differentially methylated CpG islands on the inactive X to identify sequence features that may contribute to the X inactivation process. As our study included Turner syndrome patients with single X chromosomes of both maternal and paternal origin, we searched for parent-of-origin specific methylation indicative of imprinting (Skuse, *Nature* 387:705), but were unable to find any. Our study provides the first complete epigenetic profile of X-inactivation, giving novel insights into the phenomenon of dosage compensation.

P11.141 ZNF397, an interphase specific novel mammalian centromere SCAN-zinc finger protein.

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Our laboratory has previously identified a novel centromere protein, ZNF397, using a centromere-positive autoimmune serum from a patient with watermelon stomach disease. ZNF397 protein belongs to the classical Cys₂His₂ group of the zinc-finger protein superfamily, which is one of the largest families in the human proteome. It contains two conserved domains; a leucine-rich SCAN domain and nine Cys₂His₂ zinc fingers. Bioinformatic analysis shows that ZNF397 is conserved in placental mammals. To date, only a few proteins containing zinc-finger domains have been identified at the centromeric or pericentric loci in various eukaryotic organisms.

Stable GFP-ZNF397 human cell lines demonstrated that ZNF397 is centromeric and it co-localises with constitutive centromere protein CENP-A during interphase to early prophase of human cells. Deletion and domain-swap constructs indicate that the SCAN domain is necessary, but not sufficient, for centromere localisation. ZNF397 also localises to an active neocentromere, not the inactive α -satellite centromere on a pseudo-dicentric neocentromere chromosome. Knockout studies in mice have revealed that ZNF397 is not essential for chromosome segregation or development.

The presence of ZNF397 in interphase cells but not on mitotic chromosomes suggests that it is not a constitutive structural protein and that it is unlikely to be directly involved in microtubule capture, mitotic segregation, or cytokinesis. An attractive hypothesis is that ZNF397 plays a role in the regulation of transcription at the centromere.

P11.142 MicroRNA analysis using RNA extracted from matched formalin-fixed paraffin-embedded (FFPE) and fresh frozen samples on SOLiD™ system

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Archived formalin-fixed paraffin-embedded (FFPE) specimens represent excellent resources for biomarker discovery, but it has been a major challenge to study gene expression in these samples due to mRNA degradation and modification during fixation and processing. MicroRNAs (miRNAs) regulate gene expression at post-transcriptional level and are considered as important regulators of cancer progression. Next generation sequencing technologies such as SOLiD™ provide an ideal method for measuring the abundance of miRNA molecules in different cancer stages and provide insightful information on tumorigenesis. However, currently there is no good method to systematically study miRNA expression in FFPE samples on next generation sequencing platforms.

We have designed and developed a ligation-based miRNA detection method to capture small RNA sequences in FFPE samples and convert them into templates suitable for sequencing on the SOLiD™ System. Total RNA was isolated from matched lung cancer and breast cancer FFPE and snap frozen tissues using Ambion RecoverAll™ kit. Enriched small RNA from these samples was used for library preparation, followed by sequencing on SOLiD™ system. Our results show

that small RNA extracted from FFPE samples was successfully converted to small RNA libraries. The expression profiles from FFPE and fresh frozen samples were in good correlation, suggesting that miRNA molecules are less affected by sample degradation and RNA-protein crosslink. This study provides a foundation for miRNA expression analysis on SOLiD™ system using FFPE samples in cancer and other diseases.

P12 Molecular basis of Mendelian disorders

P12.001 Whole mitochondrial genome screening in two families with hearing loss: detection of a novel mutation in the 12S rRNA gene

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Sensorineural hearing loss has been described in association with different mitochondrial multisystemic syndromes, often characterized by an important neuromuscular involvement. Until now, mutations in mitochondrial DNA, specially in the 12S rRNA, the tRNA_{Ser(UCN)} and the tRNA_{Leu(UUR)} genes were implicated in syndromic or non-syndromic hearing loss either as a primary cause or as predisposing factors. In this study, we performed a whole mitochondrial genome screening in two unrelated Tunisian families with inherited hearing loss in which we did not find nuclear mutations in the *GJB2*, *GJB3* and *GJB6* genes. Results showed the presence of a novel homoplasmic mutation in the mitochondrial 12S rRNA gene in the two probands of these two families who belong to two different haplogroups: L3 and H6a1. The m.735A>G mutation affects a conserved nucleotide of the mitochondrial 12S rRNA gene in primates and other species and had a conservation index of 78.5 % (11/14). We also detected known polymorphisms and 6 novel mitochondrial variants (m.4313T>C (MT-TI), m.8649A>G (MT-ATP6), m.9329G>A (MT-CO3), m.9467T>C (MT-CO3), m.10308C>T (MT-ND3), m.11926A>G (MT-ND4)). This study described a new mitochondrial mutation associated to hearing loss and confirmed that the mitochondrial 12S rRNA gene is a hot spot for mutations associated with hearing impairment.

P12.002** Characterization of the genetic defect underlying an autosomal recessive leukodystrophy

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An Israeli-Bedouin kindred presented with an autosomal recessive phenotype of convulsions near birth, mental retardation and cerebral palsy. Brain MRI demonstrated significant reduction in white matter and agenesis of corpus callosum. Linkage analysis ruled out association with twelve genes known to be associated with inherited defects of white matter or agenesis of corpus callosum. Using 250K SNP microarrays, a region of homozygosity on chromosome 1p33-1p32.3 was identified and further narrowed down using polymorphic markers to 7.5 cm (7 Mb) between *D1S2824* and *D1S200*, with a maximum two-point LOD score of [Zmax] = 4.39, recombination fraction [θ] = 0.0 at *D1S2748*. Six candidates of the 62 genes in the linkage interval were sequenced, identifying a novel missense mutation, C307T, in *DHCR24*, resulting in substitution of arginine to cysteine at amino acid 103 (conserved exposed residue) within the flavin adenine dinucleotide (FAD) binding domain.

DHCR24 (24-dehydrocholesterol reductase) encodes an FAD-dependent oxidoreductase expressed in the endoplasmic reticulum membrane, which catalyzes the reduction of the delta-24 double bond of sterol intermediates during cholesterol biosynthesis. Missense mutations in this gene have been associated with desmosterolosis. Plasma sterol quantification in two affected individuals demonstrated a normal

cholesterol level, but ~300-fold increased levels of desmosterol providing deficient activity of 24-dehydrocholesterol reductase.

The novel *DHCR24* mutation we describe leads to excessive desmosterol accumulation much beyond that previously described, and a unique severe novel clinical phenotype.

P12.003 A recurrent homozygous deletion in *ADAMTSL4* in isolated ectopia lentis

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To date, only two different homozygous mutations in *ADAMTSL4* have been reported in patients with isolated ectopia lentis, each mutation in one consanguineous family.

We report five individuals from four non-consanguineous and non-related families with isolated ectopia lentis. All of them have the identical homozygous *ADAMTSL4* mutation not reported so far: 20 bp of coding sequence are deleted within exon 6 (NM_019032.4:c.759_778del20). In a screen of 360 ethnically matched unaffected individuals, we found two further heterozygous mutation carriers.

This deletion is flanked by a perfectly matching 8 bp direct repeat as well as two perfect DNA polymerase α frameshift hotspots, suggesting a repeat mediated recurrent mutation event. However, the deletion always comes along with the same SNP-haplotype in all individuals (affected as well as carriers), suggesting a single founder. These findings might best be explained by a "mixture" of both scenarios: a repeat mediated mutation due to the local DNA sequence environment occurred in a small number of individuals, i.e. there is a small group of different founders for the very same mutation.

Our results further support the association of *ADAMTSL4* null-mutations to isolated ectopia lentis. Screening of *ADAMTSL4* should be considered in all patients with isolated ectopia lentis, with or without family history. In patients from non-consanguineous families, we propose a two-step diagnostic approach starting with an examination of exon 6 before sequencing the entire coding region of *ADAMTSL4*.

P12.004 Mutation analysis in families with autosomal dominant polycystic kidney disease (ADPKD) in Czech Republic

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ADPKD is the most common hereditary renal disease. The progressive formation and enlargement of renal cysts causes the decline in renal function.

The disease is caused by mutations of *PKD1* (in 85 %) and *PKD2* (in 14 %) genes. Protein products of these two genes polycystin-1 and polycystin-2 may interact with each other forming a large membrane-associated complex which has a crucial role in the regulation of cell proliferation, differentiation and normal renal tubulogenesis. The identification of mutations in genes could help to find the functional important areas of polycystins and thus reveal the molecular mechanism of pathogenesis of this disease. Both *PKD* genes are high variable; 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 mutation analysis of *PKD1* gene in our laboratory has so far detected mutations in 37 families. Only the mutation p.Arg4021X was detected in two families; other mutations are unique for individual families; 29 mutations are unique for Czech population. The mutation analysis of *PKD2* gene has so far detected mutations in 30 families. The mutation p.Pro68fsX23 was identified in 9 families and mutation p.Gln160X in 5 families; 12 mutations are unique for Czech population. Determination of localization and type of mutations within the *PKD* genes and their genotype-phenotype correlation in ADPKD families improves DNA diagnostics together with the assessment of the clinical prognosis of patients.

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P12.005 Comprehensive approach to the diagnosis of Alpha Thalassemia in Iran

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Thalassemias are a group of anemia-like blood disorders that occur most commonly in people with Mediterranean, African American and Asian backgrounds. These conditions can range from mild to life threatening. Over the last 18 years more than 3000 families with a history of affected individuals with thalassemia have been referred to our center. Most of them were Beta-thalassemia patients and significant numbers of them had normal hemoglobin A₂ level indicating they were either Beta silent or Alpha thalassemia carriers.

Since 7 years ago that alpha thalassemia investigations were established in our laboratory, variety of approaches and methods have been set up to analyze these individuals such as Gap PCR, hybridization assay, Alpha1 and Alpha2 globin gene direct sequencing.

The most common alpha thalassemia deletion among our patients was -alpha^{3.7} (44.9%). The next four most frequent deletion/mutation(s) were poly A2, -alpha^{4.2}, alpha^{IVS-1,5 nt} and --MED with frequencies of 18.2, 9.1, 6.5, and 4.3%, respectively. Five other mutations, alpha^{del19(GCG-GC)} (4.0%), alpha^{CS} (3.3%), -(alpha)^{20.5} (2.1%), Hb Adana (1.8%), and poly A1 were seen in frequencies above 1%, while the remaining 11 mutations comprised 0.4% each. Other novel mutations for alpha thalassemia such as alpha^{del9}, and 3'UTR nt46 have also been identified. We have also identified few individuals with beta silent mutations. At present we are able to perform carrier detection and prenatal diagnosis for over 99 percent of referral cases.

P12.006 Alpha-thalassemia deletions found in Romanian patients with alpha-thalassemia

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Alpha-thalassemia is a hereditary hemoglobin disorder caused by defects in the alpha-globin genes. The majority of the mutations involved in alpha-thalassemia are extended alpha globin gene deletions.

The purpose of our work was to implement a molecular diagnosis approach for patients with suspicious diagnosis of alpha-thalassemia. Patients with modified levels of cell blood count (RBC, MCV, MCH), levels of HbA2 1-2.9% and excluding other causes of hypochromic anemia were tested for the most common alpha-thalassemia deletions. DNA samples extracted from peripheral blood of all 43 subjects included in the study were genotyped using the GAP-PCR molecular method. Our results showed ten patients with modifications in the alpha-globin genes, as following: three patients are carriers of the 3.7kb deletion and in four patients was identified the MED I deletion. An interesting finding was the identification of alpha triplicated status (ααα=anti 3.7kb) in 3 patients. In one of these, alpha triplicated status is associated with cd 8 (-AA) β-thalassemia mutation resulting in a thalassemia intermedia phenotype.

In conclusion, our study shows that Mediterranean alpha-thalassemia deletions -3.7 kb and MED I are quite frequently found in alpha-thalassemia cases from our country and it is the first report about alpha-thalassemia in Romania. This approach could be a very efficient application in prenatal diagnosis for a thalassemia prevention programme in our country.

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P12.007 The genetic aspects of hereditary angioedema in Russia

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Hereditary angioedema (HAE) types I and II are the chronic disease concerning group of primary immunodeficiencies, connected with the qualitative or quantitative genetically determined defect of the genes coding synthesis esterase inhibitor of a complement component C1

(C1- inhibitor), are shown in recurrent angioedema of a skin and mucous membranes. The HAE prevalence is 1/10,000-150,000. The genetic aspects studying of hereditary angioedema will allow to carry out differential diagnostics angioedema, including pre-symptomatic diagnostics, and to optimize the treatment in Russia. In present work for the first time in Russia the mutation analysis in SERPING1 (C1NH) gene, coding C1- inhibitor, at the patients with recurrent angioedema, was performed.

A pilot sample included 6 patients. DNA sequencing of the entire coding region and exon-intron junctions in SERPING1 gene was carried out in all patients. The mutations were found in 5 patients: three mutations at patients with HAE type I (c.623_624insA, c.706T>G and c.1249+2T>G) were novel, two mutations at patients with HAE type I and II (c.578T>C and c.614G>A) were found earlier among patients from Spain and USA. The first results indicate high efficiency of SERPING1 gene sequencing for HAE types I and II molecular genetic diagnostics. In one patient with HAE types I a mutation in coding region of SERPING1 gene was not found. In publications the rare mutations in regulatory gene areas and long deletions, not revealed by sequencing, are described. To increase efficiency of molecular genetic diagnostics we schedule to design analyzing systems for those mutations.

P12.008 Apoptosis resistance following DNA damage in Ataxia Telangiectasia and Nijmegen Breakage Syndrome cells is conferred by a novel defect in mitochondrial p53 accumulation

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We have previously shown that whereas T-cells from normal individuals accumulate high amounts of total p53 and undergo apoptosis when treated with the genotoxic agent Actinomycin D (ActD), those from Ataxia Telangiectasia (AT) and Nijmegen Breakage Syndrome (NBS) patients resist ActD-induced apoptosis. We have now found similar resistance on the part of the p53-null Jurkat T-cell line, a further evidence indicating that ActD initiates a p53-dependent, non-redundant apoptotic pathway. This prompted us to look for defective p53 accumulation by AT and NBS cells following ActD treatment. Surprisingly their total p53 level was only slightly less than that of normal T cells, though immunofluorescence and protein fractionation experiments revealed that this accumulation was highly defective in the cytosol and nearly undetectable in their mitochondria. Specific inhibition of mitochondrial p53 translocation with μ pifithrin (μ -PFT) in control T-cells reduced the apoptotic response by 86%, whereas treatment with α -PFT, which blocks p53-mediated transcription, had no effect. These data confirmed that ActD-induced apoptosis depends on a mitochondrial p53 function. Export blockade experiments showed that nuclear export is not required for mitochondrial p53 translocation.

Our results disclose an undescribed defect in mitochondrial p53 accumulation in AT and NBS T-cells that makes them resistant to apoptosis following DNA damage.

P12.009 Molecular Genetics of Autosomal Recessive Mental Retardation (ARMR) in consanguineous Pakistani families.

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Mental retardation (MR) is a complex phenotype characterized by sub average general intellectual functioning and deficiency in at least two of self-survival skills which appear during childhood. Incidence of MR is relatively high in Pakistan because of high consanguinity. Autosomal recessive form of MR (ARMR) is genetically heterogeneous with eight loci and six genes reported. In the present study, we identified seven large consanguineous Pakistani families with autosomal recessive mode of inheritance having multiple affected births with MR. Purpose of the study was to improve the understanding on protective measurements for ARMR.

Nucleic acid (DNA) was extracted and subjected to STS marker analyses for mapping of homozygosity in known genes and known loci regions. All families were excluded for all known regions except MR6 (consists of three loops), which seems to be linked with PRSS12 gene.

Sequencing was performed for PRSS12 but no mutation was found. Further its two loops were subjected to the Genome wide scanning by using SNP6.0 array for detection of homozygous regions. No common homozygous region was found for both loops while five homozygous regions were found for a single loop. By checking genotypes for these two loops, no match was found. It made the molecular genetics of MR6 very complicated and we have to consider several aspects for MR6 including compound heterozygosity, more than a single gene and its clinical features. One probability for MR6 can be the novel syndromic ARMR with several genes. All these observations for MR6 shows the complexity of ARMR.

P12.010 Genetic linkage analysis in a cohort of Iranian families with autosomal recessive non-syndromic hearing impairment

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Hearing impairment (HI) is the most frequent sensory birth defect in human. Autosomal recessive non-syndromic HI (ARNSHI) is the most common type of hereditary HI. Over 70 loci are known, in which more than half of the corresponding genes have been identified. Consanguinity is a highly accepted custom in Iran and therefore the Iranian population offers an opportunity to study different AR disorders, including ARNSHI. In 31 ARNSHI families *GJB2*, the most common cause of HI, was excluded. Fifteen known ARNSHI loci were analyzed by homozygosity mapping. Later, families with S-Link values ≥ 3.3 , excluded for the 15 known AR loci, were analyzed by Illumina 6K chips (Linkage IVb panel).

Ten families were found to be linked to 6 known loci by homozygosity mapping: DFNB4 (4 families), DFNB63 (2 families), DFNB7/11 (1 family), DFNB9 (1 family), DFNB2 (1 family) and DFNB21 (1 family). Five out of the 31 families studied were qualified for a whole genome scan, 3 of which showed significant linkage based on the SNP array outcomes. The linked regions in the first and second families overlap with 2 known deafness loci, with no gene identification yet, while the third family presents linkage to a new genomic region. Further analyses were performed for the fine mapping of the linked regions to narrow down the boundaries of the loci and increase the chance of finding the corresponding disease-causing genes. DNA sequencing of candidate genes in all 3 families is going on.

P12.011 The power of array-CGH in diagnosing rare autosomal recessive nonsyndromic mental retardation. The example of *TUSC3*.**

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Mental retardation (MR) is the most frequent handicap in children, affecting 2% of the general population. Despite recent advances, the causes of nearly 40% of MR remain unclear. Although more than 60 X-linked MR genes have been identified, only six genes (*PRSS12*, *CRBN*, *CC2D1A*, *GRIK2*, *TRAPPC9* and *TUSC3*) have been implicated in nonsyndromic autosomal recessive MR (NS-ARMR). These genes are involved in different pathways, namely synaptic proteolysis, regulation of mitochondrial energy metabolism, regulation of I-kappaB kinase/NF-kappaB cascade, induction of long-term potentiation and N-glycosylation, underlying the extreme heterogeneity of pathological mechanism involved in NS-ARMR. *TUSC3* encodes a subunit of the ER-bound oligosaccharyltransferase (OST) complex that catalyzes a pivotal step in the protein N-glycosylation process. Only one frameshift mutation and a large 120-150 Kbp deletion have been previously reported in two consanguineous families. By high resolution 105K CGH-

array, we identified a homozygous 177-238 Kb duplication, including exons 2-6 in *TUSC3*, in two sibs with severe MR from consanguineous parents. Affected patients presented with normal mensurations, dysmorphic features similar to previously reported cases, moderate to severe MR with insufficient speech and normal walk, epilepsy in the female patient, behavioural disturbances in the male patient, and without any malformation. This observation confirms the implication of *TUSC3* in NS-ARMR, the expanding clinical spectrum of CDG syndromes to NSMR and the importance of the high resolution of CGH-array in the identification of intragenic rearrangements of genes implicated in MR and rare diseases.

P12.012 Congenital sick sinus syndrome caused by a novel homozygous mutation in the SCN5A gene

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Sick sinus syndrome (SSS), caused by sinus node dysfunction, is characterized by inappropriate sinus bradycardia, sinus arrest, or chronotropic incompetence. The disorder, which presents with syncope, presyncope and dizziness, often requires cardiac pacing. Familial SSS is known to be inherited in either autosomal dominant; caused by mutation in the HCN4 gene, or autosomal recessive; caused by mutation in the SCN5A gene.

Here we present the molecular finding in a large inbred family with SSS. The index case, a 1 year old boy, presented with a history of frequent attacks of syncope. Electrocardiogram (ECG) revealed sinus bradycardia and 2nd degree atrioventricular block. Four of his siblings had a similar presentation and all of them underwent cardiac pacing. The ECG in their consanguineous parents was unremarkable. Sequencing the entire coding region for SCN5A revealed a novel homozygous substitution (c.5548T>C) leading to replacement of cysteine by arginine at position 1850, which was not detected in 100 ethnically matched controls.

In this work, we report a novel homozygous SCN5A mutation in congenital SSS. The in vitro biophysical characterization of the mutant, which is in progress, may provide insight into the molecular pathogenesis of the recessive form of SSS.

P12.013 Molecular investigation of *TBX1* gene in individuals with Asperger syndrome

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Asperger syndrome (AS) is a neurodevelopmental disorder from the Autism Spectrum Disorders. It is characterized by impairments in socialization and ritualistic and stereotypic behaviors. Despite of the description of mutations in several genes, the etiology remains unknown. Genetic and early developmental factors are considered important for its occurrence. Behavioral and psychiatric disorders are a prominent part of the 22q11 deletion syndrome. Furthermore, there is a description of one individual with AS and mutation in *TBX1* gene that is mapped in 22q11.2 region. Therefore, the objective of this study was to perform a mutation screening of *TBX1* gene in individuals with AS. We analyzed 14 subjects. Mutation screening of *TBX1* by direct sequencing detected several synonymous single nucleotide polymorphism (SNP), already reported in NCBI database. The sequence variant 1189A → C was identified in one patient. This Asn397His transversion was genotyped in a sample of 100 control individuals and it was found in 20% of them. This kind of mutation in *TBX1* gene may be more common than expected, however its relevance in pathogenesis of AS still remains to be determined considering the clinical and genetic heterogeneity of the disease and the restricted number of individuals studied.

P12.014 Ataxia-telangiectasia as model for study of heightened radiosensitivity and premature aging

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Diseases with distinct progeroid features may serve as models for natural aging. Neurodegenerative syndrome ataxia-telangiectasia (AT) show progeroid features on both the level of the entire organism and isolated cells. Heightened radiosensitivity by AT correlates with diminished DNA repair potential, and inability to form cell cycle blocks after irradiation. AT heterozygote carriage tends to be quite high, 4 to 11% in various populations. Thus it is really important to detect the levels of radiosensitivity and premature aging by AT-heterozygotes. In our study, the following makers of aging were studied in 2 different AT families: activity of SA- β -gal, presence of γ -H2AX foci, diminished amount of HP-1- γ and of methylated forms of histone H3 (3MeH3K9 and 3MeH3K27), SAHF (senescence associated heterochromatin focus) formation, structural disturbances of nuclear lamina and actine cytoskeleton in intact cells. In order to define the level of radiosensitivity, dynamics of the appearance and elimination was measured: in case of DNA breaks, by means of comet assay; in case of 53BP1 and γ -H2AX foci and p21, by method of indirect immunofluorescence; checkpoint formation by flow cytometry. Cells of AT patients and their blood relatives (heterozygote carriers of disease) were demonstrated to reveal features of both premature aging, and radiosensitivity. It should be noted that in cases of AT-heterozygotes, the level of expression of features of aging and radiosensitivity tended to be intermediate between AT patients, and healthy donors. The study was supported by Program of Basic Research of the Presidium of Russian Academy of Sciences 'Fundamental Sciences for Medicine'.

P12.015 Bak proapoptotic gene alteration in Iranian patients with Ataxia telangiectasia

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Introduction:

Ataxia telangiectasia(AT) is an autosomal recessive multisystem disorder characterized by variable immunodeficiency, progressive neurodegeneration, oculocutaneous telangiectasia, and an increased susceptibility to malignancies. This study was designed to study the role of proapoptotic BAK gene in a group of patients with AT to elucidate the possible role of these genes in progression of malignancies in this disease.

Materials and Methods:

Fifty Iranian patients with AT were investigated in this study. The entire coding regions of the BAK gene (exons 2-6) were amplified using polymerase chain reaction (PCR). The PCR products were separated by 2% agarose gel electrophoresis, and all positive samples were verified by direct sequencing of PCR products using the same primers used for PCR amplification, Big Dye chemistry, and Avent 3100 Genetic Analyzer following the manufacturer's instructions (Applied Biosystems). Results: Eight of fifty Iranian AT patients (16%) exhibited a C>T transition in exon 2 (c342C>T) of the BAK gene, while none of the healthy controls had such alteration (P=0.0001). Frequency of this alteration in other population (NCBI SNP Database rs2227925) was 3.9% which in our patients were higher.

Discussion:

Alteration in the proapoptotic BAK gene was found in our study, which could elucidate involvement of the mitochondrial pathway mediated apoptosis in accelerating and developing of cancers and in immunopathogenesis of AT. Such altered apoptosis in AT could play some roles in developing cancers in this group of patients.

P12.016 A new missense mutation in the transmembrane domains constitutively activates the human Ca²⁺ receptor

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Gain-of-function mutations of the calcium-sensing receptor (CaSR) have been identified in patients with familial and sporadic hypercalcicmic hypocalcaemia. We described here a new heterozygous missense mutation localized in the transmembrane domains of CaSR, carrying by a subject with autosomal dominant hypocalcaemia. Analysis of in vitro functional properties of the mutant receptor to react to extracellular calcium did not reveal the expected leftward shift in the concentration-response curve for the mutant compared to wild-type receptor, but a constitutive effect of this mutant which exhibits 100% of the wild-type receptor activity even in the absence of extracellular calcium. This finding, which has never been described to our knowledge, suggests the importance of this new mutation or of the surrounding region on the activation of the CaSR protein.

P12.017 Genetic testing for Axenfeld-Rieger Syndrome: one year experience.

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Axenfeld-Rieger Syndrome (ARS) describes a group of genetically and phenotypically heterogeneous disorders that primarily affect the anterior segment of the eye. Individuals with ARS present with a characteristic spectrum of ocular anomalies and to a lesser extent systemic malformations. One of the most debilitating features of this disorder is the increased risk of glaucoma with approximately 50% of affected individuals acquiring this progressively blinding disease. Identifying an "at risk" group allows glaucoma development/progression to be monitored and treated appropriately.

ARS is an autosomal dominant disorder with an incidence in the UK estimated at 1/250,000. There are 5 known genetic loci for ARS, situated at 4q25 (*PITX2*), 6q25 (*FOXC1*), 11q13 (*PAX6*), 13q14 and 16q24. We have recently implemented a UK Genetics Testing Network service for the detection of mutations in *PITX2* and *FOXC1*. The two stage screening procedure involves quantitative analysis using an "in house" designed MLPA kit and subsequent point mutation analysis by DNA sequencing in dosage negative cases. To date 41 patients with a clinical phenotype of ARS have been screened; four had a *FOXC1* deletion and one had a deletion of *PITX2*. A total of eight point sequence changes were identified in both genes, including 5 novel variants. *In silico* investigation was also performed for the novel findings. Here we present our results and discuss our conclusions regarding testing and the significance of the genetic testing for this syndrome.

P12.018 MLPA analysis for molecular diagnosis of Beckwith-Wiedemann syndrome

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Beckwith-Wiedemann syndrome (BWS) is an overgrowth disorder, characterized by tissue and organ hyperplasia, macroglossia, developmental abnormalities and an increased risk of embryonal tumours (mostly Wilms tumour). The disease is multigenic, due to dysregulation of the expression of imprinted genes in the 11p15 chromosomal region. BWS is mainly caused by genetic and epigenetic abnormalities. Abnormal demethylation of the maternally methylated KvDMR1 region (DMR-differentially methylated region), as well as hypermethylation of the normally unmethylated maternal H19 gene have been described in BWS patients. The molecular diagnosis of BWS is currently very difficult and sophisticated. Here we present the successful application of methylation sensitive multiplex ligation-dependent probe amplification (MS-MLPA) protocol for molecular diagnosis of BWS. The method is divided in two steps - copy number test, which permits deletions/duplications assessment and methylation test, which gives the possibility to analyze methylation status of KvDMR1 and H19 gene.

Four families were referred to our laboratory with clinical diagnosis of BWS. The MS-MLPA analysis showed demethylation in KvDMR1 region in one family, loss of methylation in KvDMR1 region in the second family and H19 gene hypermethylation in the third family. One family

did not show abnormal methylation in KvDMR1 region and H19 gene. The molecular analysis confirmed the diagnosis of BWS in 3 out of 4 families tested. The MS-MLPA proved to be a powerful method in detection abnormal methylation profile, which is essential in clarifying the diagnosis of BWS.

P12.019 A recessive form of Best's vitelliform macular dystrophy caused by a novel mutation in *VMD2* gene

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Vitelliform macular dystrophy (*VMD2*, OMIM#153700) or Best disease is an early-onset autosomal dominant retinal dystrophy, associated with a reduced or absent electro-oculogram (EOG) and large deposits of lipofuscin-like material in the subretinal space. This disorder is progressive and loss of vision may occur. Mutations in *VMD2*, which encodes the protein bestrophin, have been associated with Best disease. Clinical features in patients with mutations in *VMD2* seem to cluster into at least four major categories that include a recently described autosomal recessive bestrophinopathy (ARB, OMIM#611809). We characterized the phenotype in a consanguineous family with ARB and a novel mutation in *VMD2* gene.

Four family members were examined clinically regarding age, best corrected visual acuity test, fundoscopy, electro-oculogram (EOG), electroretinogram (ERG), fluorescein angiography (AGF), optical coherence tomography (OCT) and fundus autofluorescence. All 11 exons of *VMD2* were amplified by PCR and mutation analysis was carried out sequencing these PCR products.

The siblings showed a novel homozygous *VMD2* gene missense mutation p.R130S, (c.388C>A) in exon 4, and the parents were heterozygous for the same mutation. The siblings had multifocal vitelliform lesions, both presented reduced ERG and preserved good visual acuity. The girl suffered vitelliform material reorganization with the right eye showing a pseudohypopion type, and left a retractile-atrophic type. The boy suffered vitelliform disc regression and also had abnormal EOG.

Our study demonstrates the usefulness of *VMD2* mutational analysis in the diagnosis of bestrinopathies. A novel homozygous mutation in *VMD2* is associated with ARB.

P12.020 Further evidence of genetic heterogeneity of CACD disease associated with inherited drusen in a large consanguineous Tunisian family

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Central areolar choroidal dystrophy (CACD) is a rare inherited disease which causes progressive profound loss of vision in patients during their 4th decade. It is characterized by atrophy of retinal pigment epithelium, photoreceptors and choriocapillaris. As the locus responsible for CACD disease is PRPH2 gene or CACD locus, we checked if both loci are involved in a large Tunisian family.

A multiple consanguineous family originating from the North of Tunisia was studied. The family included twenty one affected individuals in three living generations, six of whom presented with drusen. A linkage analysis was performed using microsatellite markers flanking the PRPH2 gene and encompassing the CACD locus in 17p13.3 locus. Peripherin gene was screened by direct sequencing.

The parents were unaffected in this family and both sexes were affected in the siblings suggesting an autosomal recessive inheritance of CACD. Linkage analysis and mutational screening showed an exclusion of linkage to both PRPH2/RDS gene and CACD locus in this family.

This first molecular study of CACD in Tunisian families gives further evidence for genetic heterogeneity of central areolar choroidal dystrophy.

P12.021 The age of mutation c.550delA in CAPN3 gene.

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Limb-girdle muscular dystrophy, type 2A (LGMD2A, MIM 253600) is considered the most frequent autosomal recessive MD almost everywhere in the world. The causative gene is CAPN3 located in chromosomal region 15q15.1-q21.1 and encoding a muscle-specific protease, calpain 3. Sixty nine unrelated Russian patients of LGMD were screened for CAPN3 mutations by direct sequencing and SSCP. In 28 patients (40.5%), eighteen already known and five earlier not described (c.106delG, c.1000delC, c.1128G>A, c.1557delCT, c.1915+10C>T) mutations were detected. In 3 patients mutation was detected only in one allele. Mutation c.550delA was found in 20 of 28 patients (71.4%) in homozygous (4 cases), compound heterozygous (14) or heterozygous (2) state, thereby in 42.9% (24/56) of affected alleles. In this work to estimate the age of the c.550delA mutation we were analyze seven microsatellite markers (D15S994, D15S968, D15S514, D15S778, D15S779, D15S780 and D15S659) flanking the CAPN3 gene in 19 chromosomes bearing the mutation and in populations chromosomes (96). In all families cases equal haplotype was found for D15S514, D15S779, D15S780 markers. The equal haplotype for more markers (D15S968-D15S514-D15S779-D15S780-D15S778) was found on six chromosomes bearing the mutation. The frequencies of various "founder haplotype" decay derivatives were used to estimate the original time of the spreading of the c.550delA mutation. The mean age was equal to 64 generations for Russian population. Assuming a mean generation length of 30 years, the time since origin would be about 1900 ± 300 years.

P12.022 Mitochondrial DNA tRNACys mutation in a family with Frontotemporal Dementia and Parkinson's disease

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Mitochondrial dysfunction has been implicated in the pathogenesis of some neurodegenerative diseases, including Parkinson's disease (PD) and Frontotemporal dementia (FTD), because of the essential role of mitochondria in energy metabolism and apoptosis. FTD is the second most common type of primary degenerative dementia. Some patients present an overlap between PD and FTD both in neuropathological and clinical aspects. This may suggest a similar physiopathology and an involvement of mitochondrial DNA in FTD, as it has been associated to PD. In order to explore whether mitochondrial mutations contribute to the susceptibility of these diseases, we analysed an Italian family with parkinsonism and FTD.

Genomic DNA was isolated from whole blood and the entire mitochondrial gene was amplified by PCR and sequenced. We also screened the proband of the family for PINK1, DJ-1, LRRK2, PGRN and TAU genes and we excluded mutations in these genes.

From the sequencing of the entire mitochondrial genome, we identified a G5783A homoplasmic mutation, already reported in literature. This mutation was identified in the proband (FTD + PD), in his brother (FTD), in his maternal uncle (PD), in his mother, in his sister but not in his father. Restriction enzyme digestion revealed absence of the mutation in 50 controls. This mutation occurs at the T arm of tRNACys, resulting in the disruption of the stem structure, which may reduce the stability of the tRNA. In conclusion, we provided further evidence of the involvement of mitochondrial DNA variation in PD and FTD.

P12.023 Mutation screening of RyR2 gene for confirming catecholaminergic polymorphic ventricular tachycardia in cases of sudden unexpected deaths.

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Introduction: Significant percentage of sudden cardiac death (SCD) below the age of 40 years has a genetic background, mainly due to the long QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT). CPVT is an arrhythmogenic disease characterized by stress- or exercise-induced ventricular arrhythmia, syncope, or early sudden death. The cardiac ryanodine receptor (RyR2) gene (chromosome 1q42-q43) has been identified as a gene responsible for CPVT inherited as an autosomal dominant trait. The RyR2 gene, which encodes the cardiac Ca²⁺ release channel, is localized across the sarcoplasmic reticulum (SR) of cardiomyocytes. RyR2 plays a crucial role in the excitation-contraction coupling in cardiac muscles. There are over 70 human RyR2 mutation discovered so far that are clustered in several discrete regions of the polypeptide, which are important for the modulation of channel function. This work aims to discover the occurrence of CPVT among low-age deaths by examination on DNA level. Method: Mutation screening of RyR2 gene is performed on genomic DNA samples extracted from both myocardium of death subjects and peripheral blood of the relatives. PCR amplified fragments covering areas with known mutations were analyzed by sequencing. For mapping of the deletions RYR2 exons, we use probes for multiplex ligation-dependent probe amplification (MLPA) analysis.

Conclusion: This methodical approach enable us to identify CPVT causal RyR2 mutations. The cause of death determination and examination of relatives enable the recognition of CPVT, and to the relatives, it will offer the chance of appropriate sudden cardiac death prevention.

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P12.024 Genetic analysis of CCBE1 in Generalised Lymphatic Dysplasia

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Generalised lymphatic dysplasia is a congenital disorder characterised by extensive peripheral lymphoedema with visceral involvement. In some cases it presents in utero with hydrops fetalis. Autosomal dominant and recessive inheritance has been reported. We have recently reported a pathogenic homozygous mutation in the CCBE1 gene in a patient with extensive, autosomal recessive, generalised lymphatic dysplasia and clinical features consistent with Hennekam syndrome. Alders *et al* reported CCBE1 mutations in 5/22 patients with Hennekam syndrome; a syndrome characterised by widespread lymphoedema. Recent studies of *ccbe1* in zebrafish have shown this gene to be required for lymphangiogenesis and venous sprouting. The zebrafish mutant, full of fluid (*fof*), showed very severe oedema.

Mutation screening of the CCBE1 gene was carried out in

1. Twenty four patients with various presentations of generalised lymphatic dysplasia (this included patients with visceral involvement such as intestinal lymphangiectasia, pulmonary lymphangiectasia, pleural effusions and pericardial effusions).

2. Twenty patients or fetuses with non immune hydrops fetalis.

No novel variants were identified in patients of either group. These results indicate that CCBE1 is responsible for causing generalised lymphatic dysplasia in only a small proportion of patients. Further studies elucidating the pathway in which CCBE1 has a role and its interactions with other genes, will possibly enable the identification of other candidate genes contributing to the pathogenesis of generalised lymphatic dysplasias

P12.025 The importance of PHOX2B sequence analysis and family studies in Congenital Central Hypoventilation Syndrome; primarily a polyalanine repeat disorder

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Congenital Central Hypoventilation Syndrome (CCHS) is a rare, life threatening condition, resulting from abnormal autonomic control of

breathing. Other autonomic nervous system abnormalities, including Hirschsprung disease and neuroblastoma, may feature in severe cases.

The paired-like homeobox gene *PHOX2B* is disease-defining in CCHS. Approximately 90% of individuals are heterozygous for expansions of a 20-residue polyalanine repeat tract, with affected alleles containing 24 to 33 alanines. Most expansions occur *de novo*, but mildly affected or asymptomatic parents mosaic for the mutation or fully heterozygous, are not rare. The remaining cases have missense, nonsense or frameshift mutations.

Bristol has provided a specialist CCHS UKGTN service since 2005, supported by local clinical expertise in genetics, paediatrics and respiratory medicine, and has identified 54 cases in total. 48 probands have polyalanine expansions, including affected non-identical twins. In 30 families with expansion mutations where both parents were available for testing, 4 parents (13%) were somatic mosaics and 2 (7%) were fully heterozygous for the expansion, one showing non-penetrance. 13-20% UK cases are therefore not *de novo* and potentially recurrent. Six probands have non-polyalanine-repeat mutations. Five have frameshift mutations (two novel, c.721_739del and c.861dupT); where detailed clinical information was provided, all these patients had a severe neonatal onset with intestinal aganglionosis. We present a Dutch pedigree in which father and son are both symptomatic with variable severity; both have the point mutation c.419C>A (p.Ala140Glu). This and other cases from the Bristol cohort illustrate the importance of family studies in this intriguing disease, and further establish genotype/phenotype correlations.

P12.026 A new alternative, evolutionarily conserved exon within the CDKL5 gene

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The cyclin dependent kinase-like 5 gene (CDKL5, MIM 300203) is composed by 20 coding exons and encodes a serine/threonine kinase that has been associated with the early-onset seizure variant of Rett syndrome (RTT, MIM 312750) and X-linked infantile spasms (ISSX, MIM 308350). So far more than 50 mutations, including nonsense, missense, splice, frameshift and microdeletions, have been reported in patients with CDKL5-related encephalopathy, affecting quasi-exclusively girls. We report the coincidental finding of an additional 123-base pair exon within the CDKL5 gene by transcript analysis in a human fibroblast primary culture. Bioinformatic study indicated an extremely conserved sequence in vertebrates both at the nucleotide and amino acid levels (>95% similarity in mammals), suggesting a potential functional relevance. Curiously, and unexpectedly considering the sequence conservation, no sequence homology was found with other regions of the human genome. We further constructed a plasmid DNA including the supplemental exon to investigate the cellular pattern of the protein in COS-7 transfected cells and studied the expression of the CDKL5 transcript including this new exon in mouse tissues. Although no evident difference in terms of cellular localization between the "wild-type" CDKL5 protein and the 41-aminoacid longer protein has been observed, additional experiments will be required to investigate the functional relevance of this novel, evolutionarily conserved domain. Most importantly, considering that all mutations have not been detected in atypical RTT patients, variants in this exon may contribute to the genetic basis of the remaining cases and are currently being considered for genetic screening in our laboratory.

P12.027 Syndromic Charcot-Marie-Tooth, a neglected entity?

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Background. Charcot-Marie-Tooth disease (CMT) is a peripheral neuropathy usually characterized by slow and progressive weakness in legs. The symptoms often progresses to the hands. Pes cavus is a

frequent deformity. CMT is the most common inherited disorder of the peripheral nervous system with an estimated prevalence of 1 in 2,500. CMT is a heterogeneous disorder making classification a challenge. Clinical classification is based on distribution of muscle weakness, age at onset and mode of inheritance. Later neurophysiology subdivided CMT into type 1 and 2, depending on whether the median motor conduction velocity (MCV) is less or above 38 m/s. A third form has intermediate MCV (25-45 m/s). CMT is also classified due to molecular genetic mutations. At present 43 genes are identified causing inherited peripheral neuropathies. The duplication of *peripheral myelin protein 22* (*PMP22*) is the most common cause of CMT. Other copy number variants (CNV) have not yet been assigned to the phenotype.

Methods. A girl with a CMT phenotype and some additional clinical features was referred for investigation.

Findings. Mutation analyses for the most common CMT genes were negative. Further analyses revealed the cause of her *de novo* phenotype.

Interpretation. The involved genes explain different aspects of her phenotype.

P12.028 Charcot-Marie-Tooth disease (CMT) and novel mutations in *GJB1* and *LITAF*

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Background: Charcot-Marie-Tooth disease (CMT) is the most common inherited disorder of the peripheral nervous system with an estimated prevalence of 1 in 2,500. CMT is a heterogeneous disorder making classification a challenge. Neurophysiology subdivided CMT into type 1 and 2, depending on whether the median motor conduction velocity (MCV) is less or above 38 m/s. A third form has intermediate MCV (25-45 m/s). Up to date 43 genes causing CMT have been identified.

Mutations in *Gap Junction Protein, beta-1 (GJB1) / Connexin 32 (Cx32)* on Xq13.1. is the second most common cause of CMT. Connexin proteins are arranged in hexameric arrays and form gap junctions which facilitate the transport of ions and small molecules between cells.

Mutations in the transcription factor *Lipopolysaccharide-induced TNF factor (LITAF) / Small Integral Protein of Lysosome/Late Endosome (SIMPLE)* on 16p13.3-p12 causes CMT1C.

Patients and families with CMT are regularly referred to Telemark Hospital for diagnostics and genetic counselling

Methods: Novel mutations in *GJB1* and *LITAF* discovered during the last year will be presented. Pedigrees and symptomatology will be described.

Results: Our results will be presented at the conference.

P12.029 Four novel connexin32 mutations in patients with X-linked Charcot-Marie-Tooth disease

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The CMT 1X is X-linked type of Charcot-Marie-Tooth disease, an inherited demyelinating neuropathy, associated with mutations in Cx32 gene (GJB1) coding for the gap junction protein, connexin 32.

We identified GJB1 gene mutations in 7 of 33 (21%) male probands with CMT where X-linked type inheritance cannot be excluded. These nucleotide changes were also found in 13 of their 17 relatives including all the six mothers available for testing.

Four of the seven mutations are novel and associated with the severe phenotype. Two of these four missense mutations are c.149C>G in E1 domain resulted in a serine at codon 50 being replaced by cysteine (Ser50Cys) and c.268C>A in TM2 domain (Leu90Ile). One substitution is nonsense - c.398G>A in TM3 domain which changes tryptophan 133 into stop codon (Trp133Stop). One sequence variation is a rare type of nonstop mutation c.851G>T in C-end resulted in a stop codon being replaced by leucine (Stop284Leu). New missense mutations were not detected in normal subjects. Three known mutations Y135C, V181M and E208K were also identified in this group.

CMT1X caused by GJB1 mutations is the second most common type of CMT in Belarus. Now GJB1 is routinely screened in all CMT1 families negative for 17p11.2 duplication and with no male to male transmission.

P12.030 Two splice-site mutations in the NTRK1 gene in a patient with congenital insensitivity to pain an anhidrosis: a novel mutation in intron 16

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Congenital insensitivity to pain and anhidrosis (CIPA, #256800) is a rare genetic disease characterized by the lack of reaction to noxious stimuli and anhidrosis. It is caused by mutations in the NTRK1 gene (OMIM *191315), which encodes the high affinity tyrosine kinase receptor I for Neurotrophic Growth Factor (NGF).

We present the case of a female patient referred to our Hospital in order to study her prolonged fever of unknown origin associated with oral leukokeratosis. The clinical diagnosis of CIPA was made at the age of 8-months, and was confirmed by the detection of two splice-site mutations in NTRK1. The first mutation, c.574+1G>A, already described, is located at the splice donor site of intron 5. We also found a second mutation, c.2206-2A>G, not previously reported in the literature, which is located at the splice acceptor site of intron 16. Each parent was confirmed to be a carrier for one of the mutations by DNA sequencing analysis. It has been proposed that the c.574+1G>A mutation would cause exon 5 skipping during NTRK1 mRNA splicing. By analysing mRNA from the patient, her parents, and several other relatives, we confirm this prediction and, more importantly, we provide evidence that the novel c.2206-2A>G mutation also disrupts normal NTRK1 splicing, leading to the use of an alternative splice acceptor site within exon 17. Currently, the patient is 6 years old and her psychomotor development conforms to her age (RMN, SPECT and psychological study are in the range of normality).

P12.031 COL7A1 mutation database

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COL7A1 encodes collagen VII, protein responsible for epidermis-dermis integration. Mutations in COL7A1 cause Dystrophic Epidermolysis Bullosa (DEB) - genodermatose characterized by spontaneous or mechanically induced bullous formation. There are about 520 COL7A1 mutations known, which result in either disturbance of protein formation, interaction and function or complete lack of collagen VII. Thus disease can be inherited in either dominant or recessive mode. In this paper we present the first COL7A1 locus specific database. The db project is in agreement with HGVS guidelines (den Dunnen JT, Hum Mutat. 2009 30:493-5). Using of the system is free of charge as well as logging in, which is required for submitting data. The db is aimed to gather all COL7A1 gene, protein and variants-related information, including e.g.: traditional and HGVS's nomenclature mutation name, graphic view of mutations and SNPs (DNA, RNA and protein level). Each mutation is linked to the page comprising details as: identification method, pattern of inheritance, consequence of mutation on molecular and clinical level. Data can be easily submitted by logged in users after completing detailed submission form (including both molecular and phenotypic details). The majority of information gathered in the system is based on YES/NO answers thus searching and statistic tools are expanded. COL7A1 mutation database was designed in order to unify and simplify molecular diagnostics and to collect multicenter genotype-phenotype data, which will, as we hope, serve in the future to find correlation between them. Supported by NN407171134 and NN 402233137

P12.032 Mapping of an autosomal recessive cone dystrophy to 2p12-2q12.1 in a family of five affected individuals from Oman

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A family of five affected children age ranging from 19 to 7 years have been diagnosed with cone dystrophy in the ophthalmology department

at Sultan Qaboos University hospital, Sultanate of Oman. The parents are first cousins and the disease fits the autosomal recessive pattern of inheritance. Linkage analysis was initiated using 10K SNP chip microarray on Affymetrix platform. Analyzing the genotypes in the affected individuals and by looking at the regions of homozygosity shared by the affecteds and comparing them to the parents have revealed mainly two regions of homozygosity. The first was 44 consecutive homozygous markers on chromosome 2 between markers rs953222; rs2310401 corresponding to the following cytogenetic band 2p12-2q12.1 and the second was 12 consecutive markers in chromosome 9 between rs888225; rs756777 corresponding to band 9q33.3-9q34.2. Microsatellite analysis of both homozygous regions confirmed linkage to the region in chromosome 2 and excluded the region on chromosome 9. The region on chromosome 2 overlaps with region mapped for Jalili syndrome, 2q11. Hence we undertook sequencing of the CNNM4 gene which is involved in Jalili syndrome that is described as autosomal-recessive cone-rod dystrophy and amelogenesis Imperfecta. However, the family that we identified has the cone dystrophy but not the amelogenesis imperfecta. Sequencing of the CNNM4 gene did not reveal any sequence variation from the normal sequence published in gene bank.

P12.033 A diagnostic approach to children with congenital ataxia and cerebellar atrophy

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Congenital cerebellar ataxias are heterogeneous and poorly classified conditions characterized by severe neonatal hypotonia, psychomotor delay, followed by the appearance of ataxia within the first years of life. We performed muscle biopsy in a series of patients with early-onset ataxia and neuroradiological evidence of cerebellar atrophy. Reduced levels of Coq10 were found in the skeletal muscle of 10 out of 60 patients that were screened. A novel mutation in the ADCK3/Coq8 gene (R347X) was identified in a female patient with ataxia, seizures and markedly reduced Coq10 content. In a 2.5 years old male patient with normal Coq10 levels and apparently non syndromic congenital ataxia with early strabismus, muscle morphology showed myopathic changes with autophagic vacuoles that prompted us to screen for *SIL1* gene mutations. A recurrent nonsense mutation (R111X) was identified in this patient, leading to early diagnosis of Marinesco-Sjogren syndrome. Following these studies, we think that muscle biopsy is a valuable diagnostic approach to this subgroup of genetic conditions and should be implemented in children with congenital cerebellar atrophy.

P12.034 CRYGC analysis in a family with primary congenital cataract

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Cataract is the leading cause of reversible blindness in childhood with an occurrence of 1-6/10,000 live new born. Cataracts are characterized by the location and structure of opacities, i.e. shape, size, color and refractive quality. Cataract may be an isolated anomaly or part of a syndrome. The majority of inherited non-syndromic cataracts are transmitted as an autosomal dominant trait. Mutations in the CRYG genes, which encode the main cytoplasmic proteins of the human lens, have been associated with cataracts of various appearances. The aim of the present study was to identify the disease locus for congenital cataract in a non-consanguineous family with three affected members. DNA from leukocytes was isolated to analyze the CRYGA-D cluster genes. DNA sequencing analysis of the three affected members showed a novel heterozygous missense mutation in the CRYGC gene. Analysis of the two unaffected members of the family and the normal parent showed a normal sequence of the CRYGA-D cluster genes. This mutation was not found in a group of 120 unrelated controls discarding a

possible polymorphism. In this study we describe a novel mutation in the CRYGC causative of congenital cataract.

P12.035 A new diagnostic approach on genetic deafness, first cases of congenital deafness diagnosed by custom molecular microarray (Array CGC)

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Introduction: Congenital hearing loss/deafness is the most common birth defect and the most prevalent sensorineural disorder in developed countries but currently only a minority of genes is included in genetic diagnostics. Genetic factors are considered to cause more than 50% of the cases of congenital deafness in children. Genetic deafness can be inherited, as an autosomal dominant, autosomal recessive, or X-linked recessive trait, as well as by mitochondrial inheritance. Over 400 genetic syndromes that include deafness have been described. Moreover, in some syndromes deafness may appear as the first symptom, while other pathological manifestations may have a later onset during development. Molecular testing is a vital asset to complement the differential diagnosis between nonsyndromic and syndromic hearing loss.

Method: Using a custom microarray panel (Arrays CGC - Patent Pending) that contains 312 point mutations, identified in 32 main genes involved on congenital deafness it is possible to identify the molecular basis of the most common forms, both syndromic and nonsyndromic.

Results: The samples analyzed were obtained from an already scrutinized population, so the most common genetic alterations were already excluded. We analyzed 69 cases and in 16 we detected mutations or sequence variants on CDH23, GJB2, GJB3, MYO1A, MYO7A, OTOF, SLC26A4 and WFS1.

Conclusion: The usual diagnostic approach only analyses few genes. With this approach we can drastically increase the number of genes/mutations analyzed maintaining accuracy but reducing turnaround time. This approach greatly enhances genetic diagnostics, allowing early decision-making process in patient management as well as new epidemiologic data.

P12.036 Molecular analysis of novel and known candidate genes in patients with isolated congenital heart defects

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Mutations of the ZFPM2/FOG2, GATA4, NKX2.5 genes have been associated with the pathogenesis of non-syndromic congenital heart defects (CHDs), in particular conotruncal (CTD) and septal defects. Here, we aimed to better evaluate the occurrence and the prevalence of mutations in these genes as well as in two novel candidates (*ISLET1* and *GDF1*) in a cohort of 202 individuals with different CTDs, including tetralogy of Fallot (ToF), double outlet right ventricle (DORV) and truncus arteriosus. Furthermore, we verified the possible occurrence of *GATA4* gene deletions/duplications in a cohort of 161 additional patients, including ToF, atrial and atrioventricular septal defects and Ebstein anomaly. DHPLC analysis disclosed no putative pathogenic mutation in *GATA4*, *ISLET1*, and *GDF1* genes. Three distinct ZFPM2/FOG2 missense variants (Glu30Gly, Ile227Val, Met544Ile) were identified in 3 of 178 (0.6%) with ToF and 2 of 13 (15.4%) with DORV. One known missense change (Arg25Cys) was detected in *NKX2.5* gene in two (1.1%) patients with ToF. MLPA analysis revealed that *GATA4* MLPA signals were all within the normal range values in all patients. The present results i) exclude a major contribution of *GATA4*, *GDF1*,

and *ISLET1* genes in the pathogenesis of the investigated CHDs; ii) corroborate the association between *ZFPM2/FOG2* mutations and ToF and suggest that these mutations may occur in a substantial percentage of patients with DORV; iii) confirm that mutations outside the *NKX2.5* gene homeodomain are responsible for a subset of patients with ToF; iv) exclude a major contribution of *GATA4* gene copy number variants in CHD pathogenesis.

P12.037 Analysis of the GJB2 and GJB6 genes in italian patients with nonsyndromic hearing loss: frequencies, novel mutations, genotypes and degree of hearing loss.

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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) and account for about 32% of cases. The subjects referred to our Hospital were affected by sensory neural deafness with various degrees (from mild to profound) of hearing loss (HL). Since January 2001 we analyzed 1102 patients and identified mutations in 707/2204 chromosomes. We characterized 41 different mutations and six polymorphisms in 399 NSHL subjects. Our data confirm 35delG as the most frequent *GJB2* mutation in the Italian population, accounting for about 65% of all the mutated *GJB2* alleles analyzed. We also identified five novel variants of unknown pathogenetic significance: the p.V156I aminoacid substitution, the c.-216T>C, the c.-41G>C and the c.-96G>C heterozygous changes in the 5'UTR and the c.684C>A change in the 3'UTR of the gene. The *GJB6* gene deletion, del(*GJB6*-D13S1830), which can cause HL in combination with *GJB2* mutations in trans, was identified in nine patients, while the del(*GJB6*-D13S1854) was not observed in our cohort of patients. Our study also tried to provide information about *GJB2* genotypes and degree of HL. Phenotypes of subjects carrying biallelic *GJB2* mutations show a variable intra and interfamilial degree of HL. We collected audiometric data from 200 patients with biallelic *DFNB1* mutations or with dominant mutation in *GJB2* to determine the degree of HL to correlate the genotypes with the audiological phenotypes.

P12.038 A novel truncation mutation in the GJB1 gene, causing an X-linked, Charcot -Marie-Tooth disease.

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X-linked CMT (CMTX) accounts for 10%-20% of all hereditary demyelinating neuropathies. At least five genes and loci are known to be associated with CMTX. Most common are mutations in the *GJB1* gene. The *GJB1* gene encodes the gap junction protein connexin32 (Cx32). Mutations in the *GJB1* gene result in a demyelinating neuropathy that predominantly affects motor axons. Although variable expression has been described occasionally for the same mutation, it has been previously suggested that premature truncation mutations result in a severe neuropathy. We describe a family with CMTX, due to a novel truncation mutation with a mild to moderate presentation.

The proband is a 72 y old male who complained of mild gait disturbances since the age of 20 years. By the age of 43 his gait started to deteriorate slowly. At the age of 63 he was admitted to a rehabilitation center with moderate leg weakness, mild arm weakness and bilateral drop foot. EMG studies were compatible with sensory/motor axonal neuropathy. Family history was compatible with an X linked inheritance pattern with no male to male transmission. The molecular study revealed a c.633-634insTA mutation in exon 2 of the *GJB1* gene. This novel mutation creates a frameshift, resulting in a premature stop codon (p.Leu212fsX42).

Most *GJB1* mutations cause neuropathy through loss of normal Cx32 function.

We suggest that a less severe phenotype in this family may be due to the rather distal truncation of the protein, retaining some of the normal function of the protein.

P12.039 Decreased expression of PTEN transcript level in a Cowden Syndrome patient without detectable PTEN gene mutations

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Cowden Syndrome (CS) is an autosomal dominant disease characterized by multiple mucocutaneous lesions, benign tumors and cancer predisposition.

80-85% of patients with CS carry detectable mutations in the PTEN gene. These mutations cause decreased protein expression, raising the hypothesis of a haploinsufficiency mechanism underlying CS pathogenesis.

Mutational analysis of the PTEN gene, based on direct sequencing of the entire coding sequence and promoter region and on the search for deletions and duplications with MLPA, is performed at our laboratory in CS and CS-like patients to a diagnostic purpose. So far, in all patients with CS but one we have found pathogenic PTEN mutations, including two novel small deletions in exon 5 and 6.

In the only patient without detectable PTEN mutations, we performed PTEN expression analysis based on qReal Time PCR on cDNA obtained from the peripheral blood cells of the patient and Epstein Barr virus-transformed human B lymphocytes.

The analysis showed that PTEN transcript was about 40% lower compared to healthy controls, being, however, higher than in a patient harbouring a frameshift mutation predicted to induce mRNA decay. The reduction of the transcript found in this case led us to hypothesize an epigenetic mechanism altering PTEN expression, which is currently under investigation. If confirmed, this would represent a novel, and probably uncommon, mechanism of PTEN dysfunction, as a previous study on PTEN-negative CS patients failed to find decreased expression of PTEN transcript levels despite decreased protein expression, suggesting a dysregulation at protein level.

P12.040 Involvement of *LTBP4* and *FBLN5* mutations in patients with autosomal recessive cutis laxa : clinical and molecular considerations**

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Hereditary cutis laxa delineates a heterogeneous group of conditions characterized by abnormalities of the elastic fibers and presenting with loose, sagging and inelastic skin and variable systemic manifestations. Mutations in the fibulin-5 gene (*FBLN5*) cause an autosomal recessive form of cutis laxa (ARCL) characterized by severe skin laxity, pulmonary emphysema and peripheral pulmonary artery stenosis. Very few *FBLN5* mutations however, have been identified so far and the genetic defect remains unknown in a significant proportion of patients. Recently the gene encoding the latent transforming growth factor-beta binding protein 4 (*LTBP4*) was shown to be implicated in families with an ARCL phenotype. In the current study, we examined a cohort of 16 patients with ARCL. Direct sequencing of *FBLN5* in all patients identified 1 known and 1 novel mutation (p.C217R and p.E391X) in 2 probands, whereas molecular analysis of *LTBP4* in the 14 remaining probands identified 9 novel loss-of-function mutations (p.R448X, p.C617X, p.S803X, p.Q1221X, p.Q1296X, p.R1377X, c.1263delC, c.4114dupC and c.780+2T>G) and 1 known mutation (c.4127insC) in a total of 8 patients. These results show that *LTBP4* mutations are more prevalent than *FBLN5* mutations in ARCL. Phenotypic comparison between *LTBP4* and *FBLN5* mutation positive patients shows overlapping but also distinguishing clinical features, i.e. *LTBP4* mutation positive patients have more severe gastro-intestinal and urinary tract involvement. Our results also suggest *LTBP4* as the first gene to test in the molecular work up of patients with ARCL.

P12.041 Simultaneous detection of common cystic fibrosis mutations by reverse-hybridization teststrips

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Cystic fibrosis (CF) is one of the most common autosomal recessive disorders, with an incidence of approximately 1 in 3000 live births in Caucasians. CF is caused by mutations in the cystic-fibrosis transmembrane regulator (CFTR) gene, encoding a chloride channel protein. Patients with classical CF accumulate viscous mucus in the respiratory and gastrointestinal system, leading to chronic lung infections, excess salt loss, difficulties in digestion, and ultimately to a shortened life expectancy. More than 1000 CFTR mutations have been described to date, the majority being very rare or private. The most frequent mutation worldwide 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 (Cystic Fibrosis StripAssay) for the rapid and simultaneous analysis of common CFTR mutations. The assay covers 23 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. Thus a coverage of 70-93% can be obtained almost all over Europe. The test is based on multiplex DNA amplification and hybridization to teststrips presenting a parallel array of allele-specific oligonucleotide probes for each mutant and wild-type allele. The procedure is rapid, simple and convenient, accessible to automation and requires very small amounts of samples, which is of particular importance for prenatal diagnosis and newborn screening. Currently the Cystic Fibrosis StripAssay is validated in a multi-center study. (oberkanins@viennalab.co.at)

P12.042 Cystic fibrosis transmembrane conductance regulator gene mutations in patients from the Middle Black Sea region of Turkey

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Cystic fibrosis (CF; OMIM no.#219700) is the most common autosomal recessive disorder in the Caucasian population (1/2500). Disease is caused by mutations in cystic fibrosis transmembrane conductance regulator (CFTR; 602421) gene. The aim of this study was to determine the frequencies of the 36 mutations in CFTR gene in patients with CF from the middle Black Sea region of Turkey.

We screened 254 patients with suspicion of CF for CFTR mutations using a multiplex PCR based reverse hybridization assay.

Overall, CFTR mutations were detected in 13.0% of the patients, while no mutations were detected in 87.0%. F508del allele was present at 8.8%; N1303K 0.8%; I148T 1.2%; W1282X 0.8%; 2789+5G-A 0.4%; R1162X 0.4%; 1706del117 0.4%, E60X 0.4%, and dele2,3 0.4%. Of the 33 patients with CFTR gene mutation(s), 24% were homozygotes, 70% were heterozygotes with single mutations, and 6% was compound heterozygotes with two mutations.

We were able to identify 10 different allelic combinations of the 36 mutations tested in CFTR gene in Turkish patients in whom CF was suspected. The data seems to indicate that the Turkish population may have variable genetic heterogeneity at the CFTR locus.

P12.043 CFTR mutations in newborns with hypertrypsinogenemia in Russian population

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Cystic Fibrosis (CF; OMIM no.219700) is the most common severe autosomal recessive disorder in Caucasians caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The spectrum of presentation of cystic fibrosis (CF) is significantly varied. Neonatal hypertrypsinogenemia is one of the clinical symptoms due to the functional insufficiency in the pancreas. Neonatal screening for CF consists of the immunoreactive trypsinogen (IRT) test usually as the first step. The aim was to search the CFTR mutations common in Russian CF patients in newborns with the first positive IRT test result. DNA samples extracted from dried blood spots of 990 newborns with

the first positive IRT test result were analyzed for 11 *CFTR* mutations (CFTRdel2,3(21kb), F508del, I1del507, 1677delTA, 2184insA, 2143delT, 2183AA>G, 2184delA, 394delTT, 3821delT, L138insA). All newborns were born Moscow during 01.01.2008 to 31.12.2008 period. Screened mutations shared 67,5% out of all mutant alleles of CF patients from Russia. MultiplexPCR and gel electrophoresis were carried out for mutation screening. 5 persons with two *CFTR* mutations (3 - F508del/F508del, 1 - CFTRdel2,3(21kb)/CFTRdel2,3(21kb), 1 - F508del/2184insA) and 38 persons with one mutant *CFTR* allele (26 heterozygous for F508del, 5 - CFTRdel2,3(21kb), 2 - 2143delT, 2 - 2184insA, 1 - 3821delT, 2 - L138ins) were revealed during the investigation.

The total frequency of identified CF mutant alleles in Moscow newborns with the first positive IRT test result was 0.02424 (0.01883±0.03073). This evaluation is significantly higher than the frequency of these mutations in the Russian population defined previously (0.00642 (0.0041±0.00951; $\chi^2=29,26$; $p<0.001$).

P12.044 Phenotypic consequences of the ΔF508 mutation in pancreatic insufficient Iranian CF patients

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Cystic Fibrosis (CF) is the most common lethal recessive autosomal disease in European populations, with an estimated incidence of one in 2000 live births. Hundreds of mutations in the causative gene, *CFTR*, have been identified. The ΔF508 mutation is the most common mutation and constitutes approximately 70% of the mutated alleles worldwide. CF is associated with a wide phenotypic spectrum, and the spectrum of phenotypes varies in different geographical regions and amongst different ethnicities. Homozygosity of the ΔF508 mutation is generally associated with a very severe phenotype. Here, we aimed to assess the overall phenotypic effects of the ΔF508 mutation in a subgroup of Iranian CF patients exhibiting pancreatic insufficiency. Genotype/phenotype correlations of patients homozygous for the ΔF508 mutation, heterozygous for this mutation, and those not harboring a ΔF508 allele were assessed. Thirty six patients were studied. Demographic, clinical, radiologic and paraclinical data were recorded. The ΔF508 mutation was screened by an allele specific PCR protocol. Descriptive and analytical statistics of the results were performed using SPSS16 software and the χ^2 -squared test. Seven (19.4%) patients were heterozygous for ΔF508, 2 (5.6%) were homozygous, and 27 (75%) did not harbor the mutation. Occurrence of dyspnea and bronchiectasis was significantly higher in homozygous patients compared to the group without a ΔF508 allele. Amount of chloride ion in the sweat, and fat droplets in the stool were also higher in the homozygous group. No other statistically significant difference was observed among the groups.

P12.045 Mutational analysis of the *DCX* and *LIS1* genes in patients with Lissencephaly and Subcortical band heterotopia from Southern Italy

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Classical lissencephaly (LIS) and subcortical band heterotopia (SBH) or 'double cortex syndrome', are neuronal migration disorders associated with epilepsy and mental retardation. SBH is usually caused by mutations in the *doublecortin* (*DCX*) (Xq22.3-q23) gene, and much less frequently in the *LIS1* (17p13.3) gene. Both genes encode proteins involved in microtubule homeostasis. *DCX* mutations predominantly cause SBH in heterozygous females and severe lissencephaly in males, although rare males with SBH and *DCX* mutations have been reported. In this study, we have analyzed familial and sporadic patients with SBH or LIS from Southern Italy to investigate abnormalities of the *DCX* gene. We have performed a mutational analysis of this gene in 7 patients (5 sporadic and 2 familial) with SBH or LIS by direct sequencing;

ing; esonic rearrangements were carried out by multiplex ligation-dependent probe amplification (MLPA). We have found a stop codon mutation (R303X) in the exon V of the *DCX* gene in one unrelated female with SBH and we have excluded deletions and duplications by MLPA in all the patients. These preliminary data suggest an involvement of other genes besides *DCX*, unless some variants are located in the unexplored 5-untranslated region or in the promotore sequences of this gene. The analysis of the other major gene implicated in these disorders, *LIS1*, is still in progress. However, since a small percentage of genetically undiagnosed cases of lissencephaly have been reported, it would be interesting to extend our study to other genes, such as *ARX*, *TUBA1A*, *FLNA*, involved in cortical development.

P12.046 Mutational screening of the 35delG of the connexin 26 gene among Libyan Non syndromic Recessive deafness cases

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A single mutation (35delG) in the *GJB2* gene, encoding the gap-junction protein connexin 26, accounts for the majority of nonsyndromic recessive deafness (NSRD) in many different populations. This mutation represents a deletion of a guanine within a stretch of six Gs between nucleotide positions +30 and +35 of the *GJB2* cDNA (35delG). The aim of this study is to determine the frequency of this mutation among the Libyan NSRD patients. To screen for this mutation, we analyzed the genomic DNAs of 139 affected patients, originating from different parts of the western Libya and were genotyped by direct restriction enzyme analysis of the targeted amplified PCR products. Out of 139 samples, 11 samples were heterozygous for the 35delG mutation and were verified by direct sequencing. This result shows that most likely several genetic loci underlie the NSRD in Libya as 35 delG mutation were found to explain only 8% of the NSRD cases. Further analysis of the *GJB2* gene and other deafness genomic loci in Libyan patients is warranted.

P12.047 Homozygosity for IVS1+1G>A *GJB2* (connexin-26) splice site mutation in 73 unrelated patients with nonsyndromic hearing loss from East Siberia

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We studied *GJB2* mutation spectrum in patients with nonsyndromic hearing loss (NSHL) in the Sakha Republic (East Siberia, Russia). A total of 150 patients with NSHL of different ethnic affiliation (Caucasians and Asians) were analyzed by PCR-SSCP and further sequencing of *GJB2* gene. The molecular screening of IVS1+1G>A was performed using by PCR-RFLP method [Sirmaci et al., 2006], detection of del(GJB6-D13S1830), del(GJB6-D13S1854) was carried out using appropriate protocols [del Castillo et al., 2005].

GJB2 mutations (IVS1+1G>A, 35delG, V27I, M34T, V37I, 312del14, 333-334delAA, R127H, E114G) and one large deletion of 342kb - del(GJB6-D13S1830) were found in 50.9% of Caucasian and 68.9% of Asian patients chromosomes. One of the common mutation in Caucasian (Russian) patients is 35delG, in Asian (Yakut) patients is IVS1+1G>A. We found 73 unrelated patients with IVS1+1G>A mutation in homozygote state, in 18 individuals IVS1+1G>A was detected in compound heterozygote state with other *GJB2* mutations. Molecular screening of IVS1+1G>A in hearing individuals from 6 populations of Sakha Republic (Yakuts, Dolgans, Evenks, Evens, Yukaghirs, Russians) showed that it was one of the common allelic variant in Turkic-speaking populations of Yakuts and Dolgans. This is a first report of IVS1+1G>A in East Asians population. High prevalence of IVS1+1G>A in Yakut isolate population may be a result of common founder effect. This work was supported by RFBR (09-04-01123-à) and RHF (08-06-84602a/U).

P12.048 GJB2 caused hearing loss in Russia

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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 367 Russian patients with PNSRD. The c.35delG mutation frequency was most high and was found to be 79% in all mutant chromosomes. Therefore, from homozygous and heterozygous/compound heterozygous c.35delG carriers frequencies, the calculated portion of GJB2 caused deafness is to be 47%. The c.35delG mutation frequency was calculated to be 77% in GJB2 caused cases. Another mutations c.-3202+1G>A or IVS+1G>A (4%), c.313_326del14 (3%), c.235delC (3%), c.167delT (2%), p.Glu120del (1%), p.Leu90Pro (1%), p.Met34Thr (1%), p.Trp24X (1%) and found in one chromosome mutations p.Arg32His, p.Leu205Pro, p.Glu129Lys, p.Arg184Gln, c.-3224C>A, c.129delG, c.290_291insA, p.Gly200Arg, which last four are novel, were revealed at compound heterozygous or homozygous state. Five questionable sequence variations (p.Val27Ile, p.Val37Ile and novel c.*3C>A being 1% apiece; p.Val153Ile and p.Gly160Ser in 1 chromosome) was found at heterozygous state; only in one patient p.Val37Ile was compound heterozygous with c.35delG. The mutation 309 kb del in GJB6 gene (delGJB6-D13S1830) was not found in the patients with one mutant chromosome only. That, in general in GJB2 caused deafness, the portion of patients with one mutant allele only remains to be 17%, in which 12% are patients heterozygous at c.35delG, is to have been investigated.

P12.049 Spectrum and frequency of SLC26A4 mutations among Czech patients with non-syndromic hearing loss

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Mutations in SLC26A4 cause Pendred syndrome (PS) - hearing loss with goitre - or DFNB4 - non-syndromic hearing loss (NSHL) with inner ear abnormalities as Enlarged Vestibular Aqueduct (EVA) or Mondini Dysplasia (MD). We tested 303 unrelated Czech patients with early NSHL, all GJB2 negative, for SLC26A4 mutations and evaluated their clinical and radiological phenotype. Among 115 available HRCT/MRI scans we detected 3 MD (2.6%), 3 Mondini-like affections (2.6%) and 16 EVA (13 bilateral - 19.2% and 15.6% respectively), 61 HRCT/MRI scans (73.4%) were EVA/MD negative. We found mutation(s) in 26 patients (8.6%) and biallelic mutations in 8 patients (2.7%) out of 303 tested. In 18 of 26 (69%) patients with at least one mutation, no second mutation could be detected even using MLPA. The spectrum of SLC26A4 mutations in Czech NSHL patients is broad without any prevalent mutation. We detected 21 different mutations of which 4 are novel. The most frequent mutations in Czech patients are p.Val138Phe and p.Leu445Trp representing 18% and 8.9% of pathogenic alleles respectively. The other mutations have not been found more than twice. Among 13 patients with bilateral EVA, biallelic mutations were detected in 6 patients (50%). In EVA-negative patients no biallelic mutations were found but 4.9% had monoallelic mutations. SLC26A4 mutations are present mostly in patients with EVA/MD and/or progressive HL and those with affected siblings.

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P12.050 Unclear interactions of GJB2 and mitochondrial DNA mutations in families presenting non-syndromic deafness

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Mitochondrial DNA (mtDNA) mutations are present in less than one per cent of children with prelingual deafness, but more frequent at a later age. In the Caucasian population, at least 5% of postlingual, non-syndromic hearing impairment is due to known mtDNA mutations, representing the most frequent cause of hearing loss after the 35delG mutation in the GJB2 gene encoding connexin 26. MtDNA mutations usually lead to progressive hearing loss with an age of onset varying from childhood to early adulthood. Interestingly, there is a great variability of phenotype between individuals harboring the same mitochondrial mutation, even within the same family, and the phenotype may range from profound deafness to completely normal hearing. In the past years, the debate on mitochondrial mutations has been about penetrance, tissue specificity and the mechanisms of modifier genes that can modulate the severity of the phenotypic expression of the deafness-associated mtDNA mutations. A synergism between GJB2 and A1555G mtDNA mutations has been suggested and can be explained by an ability to maintain the normal turnover rate of the connexin 26 gap junction protein because of the reduced amount of ATP caused by the A1555G mutation. Here we present families originating from Spain, Japan, and Greece, all harboring one GJB2 and one deafness-causing mtDNA mutation, and we discuss the impact of the coexistence of mitochondrial and GJB2 mutations in these families.

P12.051 Low penetrance of the G7444A mitochondrial DNA deafness-causing mutation in a Greek family

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Mitochondria harbor their own DNA, known as mtDNA, encoding certain essential components of the mitochondrial respiratory chain and protein synthesis apparatus. MtDNA mutations have an impact on cellular ATP production and many of them are undoubtedly a factor that contributes to sensorineural deafness, including both syndromic and non-syndromic forms. Mitochondrial disease is characterized by an impressive degree of variation, and both inter- and even intrafamilial variation is the rule rather than the exception. The G7444A mtDNA mutation affects COI (the precursor of tRNASer(UCN)), encoding the first subunit of cytochrome oxidase. The mutation has been associated with non-syndromic hearing loss in only a few families worldwide, and has been reported alone or in cosegregation with other mtDNA mutations. In this study we describe the first Greek family with the G7444A mtDNA mutation, which to our knowledge is the first reported also harboring the GJB2 35delG mutation. The proband, his brother and their mother had the G7444A mtDNA mutation and also the GJB2 35delG mutation in heterozygosity. The phenotype of the family members carrying the two mutations was extremely variable; the proband had hearing loss and various other symptoms; his brother had speech delay but normal hearing and oral communication skills; the mother had normal hearing and speech. Our study demonstrates a low penetrance of the G7444A mtDNA mutation in this family, and indicates that a possible synergism between the G7444A mtDNA mutation and the GJB2 35delG mutation seems rather unclear.

P12.052 Molecular analysis of patients with congenital adrenal hyperplasia in Uberaba and Uberlândia region, MG, Brazil

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Deficiency of 21-hydroxylase (21-OHD) is the most common form of congenital adrenal hyperplasia (CAH), an autosomal recessive disorder. Clinical forms of CAH are divided as: classical (salt-wasting or

simple virilizing) and non-classical form. This variability is associated with mutations in the *CYP21A2* gene that in 85% of the cases are derived from its non-functional pseudogene (*CYP21A1P*). Analyses of deletions, large gene conversions and pseudogene-derived mutations in a group of patients with 21-OHD were performed. Patients are from a geographic region in Brazil different from those already studied. Molecular genotypes were obtained for 121 subjects representing 33 unrelated families. Eleven children (31.4%) presented with the salt-wasting, two (5.6%) with the simple virilizing, twenty (60%) with the non-classical, and two (5.6%) have not been classified. From a total of 21 affected alleles segregating in the patients with the classical form 19% and 14% carried, respectively, large gene conversions and deletions. Each of pseudogene-derived mutations IVS2-13A/C>G, I172N and cluster 6 was found in 9.5% and Q318X was identified in 4.8%. IVS2-2A>G and 992_993insA mutations that are not pseudogene-derived were identified in two alleles. Seven out of the 34 affected alleles in the non-classic presented the mutation V281L (20.5%). Within this group, conversion, IVS2-13A/C>G and I172N were found each in one allele (3%). Nineteen and 70% alleles remained undetermined, respectively, in classical and non-classical forms. Comparing to others studies in Brazil, frequencies for conversion and deletions are higher for the group studied here indicating that regional characteristics within the country might influence mutation frequencies.

P12.053 Novel Homozygous Mutation in *DSP* Causing Skin Fragility-Woolly Hair Syndrome: report of a large family and review of the desmoplakin-related phenotypes

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Desmoplakin is member of the plakin family of cytoskeletal linkers. Along with other proteins, plakins are crucial for the function of the desmosomes. Linking desmoplakin to certain types of cardiocutaneous syndromes has been a hot topic recently. Skin fragility-woolly hair syndrome is a rare autosomal recessive disorder involving the desmosomes and is caused by mutation in the desmoplakin gene (*DSP*). We report five members from a large family with skin fragility-woolly hair syndrome. The proband is a fourteen-year-old girl with palmoplantar keratoderma, woolly hair, variable alopecia, dystrophic nails, and excessive blistering to trivial mechanical trauma. Cardiac evaluation revealed normal heart. The skin biopsy from a blister showed variably necrotic roof and moderate spongiosis in the adjacent epidermis. Haplotype mapping and linkage analysis revealed a high LOD score of 4.7 in the region in the short arm of chromosome 6 that harbors the *DSP* gene. Full sequencing of the *DSP* gene demonstrated a novel homozygous c.7097 G>A (p.R2366H) mutation in all affected members, and the parents were heterozygous. This is the report of the 3rd case/family of the skin fragility-woolly hair syndrome in the literature. We also present a clinical and molecular review of various desmoplakin-related phenotypes, with emphasis on onset of cardiomyopathy. The diagnostic approach to this family depicts the value of linkage analysis versus the candidate gene strategy.

P12.054 DFNB1 hearing loss in Iran

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Introduction: DFNB1 is the most prevalent locus causing nonsyndromic sensorineural hearing loss in many populations. Mutations in GJB2 & 6 genes are the main cause of autosomal recessive nonsyndromic hearing loss (ARNSHL). Mutation detection is essential for carrier detection and genetic counseling of the families affected by hearing loss.

Materials and Methods: We investigated 91 patients from fifty families affected by ARNSHL. Clinical studies and genetic counseling were performed for all families. GJB2 and GJB6 genes were sequenced directly. Also, we checked three known large deletions in GJB6 gene. We also compared our results with the previous studies in Iranian populations.

Results: Frequency of GJB2 mutations was 20% in this study. Here, we report three novel mutations in GJB2 gene: 313-322delAAGTTCAT-

CA, E110D and G12D. Neither point mutations nor large deletions in GJB6 gene were found. Mean frequency of GJB2 mutations was 18% in Iranian populations.

Discussion: GJB2 and GJB6 mutations are not major causes of hearing loss in Iranian groups compared to European cohorts. We suggest other genes may be involved in our populations. However, GJB2 gene should be checked for all patients, at first.

P12.055 Most distal Renal Tubular Acidosis (dRTA) cases in Cyprus are caused by two *ATP6V1B1* founder mutations originating around 17th century AC. First prenatal diagnosis.

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Mutations in the *ATP6V1B1* gene (subunit B1 of apical H⁺ ATPase) cause a recessive form of distal renal tubular acidosis (dRTA), associated also with early sensorineural hearing loss (SNHL). In this study, nine dRTA families from Cyprus and one from Greece were analyzed for mutations in *ATP6V1B1* gene by DNA re-sequencing and PCR-RFLPs.

Results show that 78% of dRTA cases in Cyprus are caused by 229+1G>T and R157C mutations in *ATP6V1B1* gene. Both mutations originate to common founders as it has been proved by flanking microsatellite markers and haplotype analysis. 229+1G>T mutation was estimated to be older than 400- yo through Linkage Disequilibrium estimation and a specific equation. The mutations are not clustered in specific geographic regions of the island, probably due their old age. No genotype - phenotype correlation was found for these two founder mutations with relation to progressive SNHL. A known (L81P) and a novel mutation (912delT) were found in the Greek family. Prenatal diagnosis was performed for one Cypriot family, after parents demand, showing that the embryo was heterozygous carrier.

Existence of only two *ATP6V1B1* mutations in the Cypriot population is an advantage for easy and fast molecular diagnosis. The age of onset of SNHL varies in our patients and probably is not related to *ATP6V1B1* genotypes. Effective therapy for most of the syndrome symptoms is not satisfactory for some parents who choose prenatal diagnosis to ensure their child's health.

P12.056 Detection of Duchene muscular dystrophy carriers with quantitative fluorescent PCR

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Duchenne muscular dystrophy (DMD) is most common X linked neuromuscular disease. DMD is progressive, lethal and results from mutations in Dystrophin gene at Xp21.2. Partial gene deletions responsible for up to 60-65% DMD cases and 5-10% of cases result from gene duplications which are located in two "Hot spot" regions. Point mutations and insertion of few nucleotides account for the remaining cases (25-30%). Mutations are either inherited from female carriers (2/3) or occur de novo (1/3). In affected males, deletions can be easily detected using multiplex PCR. But determining female carrier status is difficult. This difficulty reflected in the great variety of techniques that have been used for this purpose, such as linkage analysis, FISH, MLPA. In our study we used gene dosage method quantitative fluorescent PCR (QF PCR). QF PCR refers to the amplification of DNA fragment using fluorescence-labeled primers, followed by quantitative analysis to determine copy number of DNA fragments. We used this method to determine deletions in 23 DMD patients, who were previously diagnosed and 24 female in carrier status. We tested 18 exons, promoter region and STR markers as internal control. We found the same results, which previously reported, in 19 patients. In 3 patients we found an extra exon deletion and in 1 patient we found less exon deletion than previously found. In 19 families females were carriers. As a result we conclude that QF PCR is a fast, reproducible robust

method for detection of deletions of DMD patients and it's useful for carrier screening.

P12.057 The first MLPA analysis of dystrophin gene duplications in Iranian DMD/BMD patients

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Duchenne Muscular Dystrophy and Becker Muscular Dystrophy can be caused by deletions, duplications or point mutations in the DMD gene. Partial gene duplications account for up to 5-10 % of DMD and up to 5- 19% of BMD cases. Cases with gene duplication in DMD/BMD are determined by quantitative methods such as MAPH, Sothern blotting and Q-PCR that are laborious and technically demanding. We have applied multiplex ligation-dependent probe amplification (MLPA) assay to simultaneously screen all 79 DMD gene exons for deletions and duplications in DMD/BMD patients. MLPA was performed using the SALSA MLPA kit P034-A2 and P035-A2 (MRC-Holland, Amsterdam, the Netherlands) based on the instruction of manufacture. MLPA samples consisted of approximately 200 ng of genomic DNA. All amplified fragments were separated using capillary electrophoresis on ABI PRISM 3130 Genetic Analyzer. The area under peak for each individual amplified fragment was measured using the gene marker software (v.1.6). Analysis of mutations was performed in 20 unrelated DMD patients previously found to be negative for large deletions by standard multiplex PCR assays. Gene duplication was found in five patients (25%) with various lengths from 4 to 22 exons across dystrophin gene. This is the first report of the relative frequency of duplicational mutations in Iranian DMD/BMD patients. The DMD MLPA test provides a simple and cheap DNA- based test for deletion/duplication screening of total dystrophin gene. This technique can follow by Real-time PCR assay to confirm the genetic defects in DMD gene and to improve the precision of genetic counseling.

P12.058 High Resolution Agilent 244K oligoarray CGH analysis of a patient with DMD and MR

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The dystrophinopathies include a spectrum of muscle disease caused by mutations in the DMD gene encoding for dystrophin. The clinical phenotype is variable, ranging from mild (muscle cramps, asymptomatic increase in creatine phosphate kinase), to severe (progressive muscle disease, classified as Duchenne - Becker muscular dystrophy). Dystrophin is expressed in skeletal muscles, cardiac muscle, retina and brain. The DMD gene has 7 known promoters, among these, Dp71 and Dp140 are particularly abundant in foetal brain, which has led to the suggestion that they may contribute to the cognitive defects in DMD. Intellectual disability is well described in association with up to 30% of cases of Duchenne, with a preferential impairment of verbal IQ. Here, we present a specific case of DMD with a tandem de novo duplication of exons 2-18 of the DMD gene (diagnosed by array-CGH and subsequently confirmed with MLPA analysis) in a patient with predicted DMD phenotype and profound microcephaly, facial dysmorphism and brain atrophy. Additional a-CGH findings that could contribute to his severe phenotype were a 3.0Mb deletion in 10q23.31- q23.33, resulting in haploinsufficiency of amongst others the FER113 gene, which has been implicated as a possible modifier of DMD phenotype and a 0.703Mb deletion of Xq21.31 containing the PCDH11X gene which plays a fundamental role in cell-cell recognition and is essential for the segmental development and function of the central nervous system. This case shows a central nervous system-specific and restrictive phenotype for a disorder that is conceptualized as being progressively neuromuscular in clinical expression.

P12.059 Further improvement in the I-PEP method for low copy number DNA profiling at forensic genetics

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DNA profiling has become one of the most robust and reliable methods at forensic identification; However, an insufficient DNA quantity (less than 100 Pg or 33 copies), found often in forensic evidence samples, is a major hindrance. Amplification of such low copy number (LCN) DNA samples, is attainable with the most efficient whole genome amplification (WGA) method, named improved primer extension preamplification (I-PEP) PCR.

By initial assessment of existing methods (i.e. PEP and I-PEP) on serially diluted DNA, it was attempted to reach an improved method leading to reliable profiling with the lowest amount of template. This method employs degenerate 15-mer PCR primers followed by specific amplification of DNA with specific primers. Subsequent to the amplification with the new modified and improved I-PEP, which we term KI-PEP PCR, complete DNA profile was obtained using only 2.5 pg of input DNA. Using this method, a fragment size of 1106 bp was effortlessly amplified with the specific primers. These results demonstrated fifty percent reduction in the template amount reported to be required by commercial DNA identification kits using the I-PEP method. In conclusion, the utility of KI-PEP PCR, not only increases the low quantity of DNA, but also provides optimum length appropriate to DNA typing.

Keywords: DNA Profiling, Forensic genetics, LCN DNA, Whole genome amplification, Improved primer extension preamplification PCR, KI-PEP PCR

P12.060 Dubowitz-Syndrome associated with Hyper-IgE-Syndrome in a female patient. No evidence thus far for a common genetic basis.

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An association of Dubowitz-syndrome and Hyper-IgE-syndrome (HIES) or Job-syndrome thus far has just extremely rarely been reported. HIES, a rare disorder, is heterogeneous with an autosomal dominant form caused by mutations of the STAT3-gene but autosomal recessive variants of HIES with at least two different causally related genes have been identified as well. Dubowitz-syndrome, a very rare disease, follows an autosomal recessive mode of inheritance. Recently a microdeletion/duplication has been reported in a patient with Dubowitz-syndrome. Here we describe another patient with an association of both syndromes. She is now 30 years old and fulfills the required diagnostic criteria for HIES and Dubowitz-syndrome as well. It was possible to demonstrate that a de novo heterozygous mutation of the STAT3-gene is responsible for her Hyper-IgE-syndrome. Cytogenetic and microarray CGH analysis using an Agilent 244 k oligonucleotide chip ruled out an aberration or genomic imbalance up to the resolution achieved. In addition an Affymetrix Genome-Wide Human SNP Array 6.0 analysis was performed for homozygosity mapping of larger genomic blocks of a possible LOH since a consanguinity of the parents of the patient is not unlikely. Several larger segments of suspected LOH of up to about > 3 Mbs which will be analyzed in more detail have been identified. This might allow narrowing down candidate gene regions for Dubowitz-syndrome however none of the larger LOH loci maps to the STAT3-gene or any other known HIES gene locus.

P12.061 Duchene/Becker Muscular Dystrophy, phenotype-genotype correlations and folate cycle genes interaction

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MTHFR, MTRR and MTR are the most important enzymes in the homocysteine and methionine metabolic cycle. The enzymes functions are to convert 5 and 10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, providing methyl radicals to homocysteine and synthesizing methionine in this metabolic process. A common (C677T) polymorphism in the MTHFR gene results in thermostability and reduced MTHFR activity that decreases the pool of methylTHF and increases the pool of methyleneTHF. Recently, another polymorphism in MTHFR (A1298C) has been identified that also results in diminished enzyme activity also as MTRR A66G polymorphism. We tested whether carriers of these variants alleles have the worst effect of the myopathic process.

We analyzed DNA of 14 patients with the Duchene muscular dystrophy. The PCR multiplex deletion test (two 9 plex mixes) are used for detection of dystrophin deletions MTHFR, MTRR variant alleles were determined by a PCR - RFLP assay.

Patients with in-frame deletions in dystrophin gene in combination with heterozygous status MTHFR, MTRR genes have severe clinical picture instead the mild. Patients with mild clinical picture have the next genotype of folate circus genes: 677TT, 1298CC.

During researches were we found out incidence of C677T(21,4%). This mutation lead to reduction activity of MTHFR with 35-60% which influence myopathic process. The proband (43 years old) with deletion of 47-48 exons and with genotype C677C and C1298C had not very severity clinical feature. Analysis of MTRR polymorphism reviled prevalence of A66G(92,9%).

Were set interaction between genes of folate circus MTHFR, MTRR, MTR and difficulty of Duchene/Becker Miodistrophy

P12.062 COL7A1 mutation spectrum in patients with Dystrophic Epidermolysis Bullosa in Poland

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Dystrophic Epidermolysis Bullosa (DEB) is genodermatose caused by mutations in COL7A1, which spans 32kb and comprise 118 exons. Its product - collagen VII, forms homotrimers which further assembles into anchoring fibrils, structures responsible for epidermis-dermis integration. Mutations in COL7A1 can result in either disturbance of anchoring fibrils formation and function or complete lack of collagen VII. There are about 520 COL7A1 mutations known, of which only few are recurrent, mainly among individual ethnic groups. We present the results of molecular analysis of 31 DEB patients of Polish origin. We found that c.425G>A is the most frequent mutation in our group of patients (42% of patients; 27% of alleles). Another recurrent mutations are: c.682+1G>A (13%; 6% respectively) and, unreported in the literature data before*, c.7154delC (10%; 5% respectively). Our analysis is still proceed, but up to now we also found 11 rare variants, including 7 changes unreported before*. In conclusion, our work is the first COL7A1 genotyping study performed in Polish DEB patients. Our results enable us to propose patients of Polish origin the molecular diagnostic scheme, which will be completed soon.

*according to PubMed and HGMD free version.

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P12.063 Analysis of the COL7A1 gene in Czech patients with dystrophic epidermolysis bullosa, novel and recurrent mutations.

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Background: Dystrophic epidermolysis bullosa (DEB) is an inherited skin fragility disorder where blistering occurs in the sublamina densa zone at the level of anchoring fibrils of the dermo-epidermal junction zone. Both autosomal dominant (DDEB) and recessive (RDEB) forms result from mutations in the type VII collagen gene (COL7A1). Ob-

jective: The purpose of this study was to analyse the COL7A1 gene and perform genotype-phenotype correlations in Czech patients with DEB. **Methods:** Mutations in the COL7A1 gene were characterised using polymerase chain reaction and DNA sequencing. **Results:** DNA analysis of the COL7A1 gene was performed in 27 probands with diagnosis of RDEB and 6 probands with diagnosis of DDEB. 29 different sequence variants were found, ten of which have not been reported previously. In the set of our RDEB patients, the most frequent mutation was the splice site mutation c.425A>G (29,6% of RDEB mutant alleles). The novel mutations comprised "silent" glycine substitutions (p.Gly1845R, p.Gly2296Glu, and Gly2557Arg), the novel nonsense mutation p.Ser609X, three splice site mutations (c.3894+1G>A, c.5856+1G>A and c.6751-2delAG), the deletion c.4556delG, the insertion c.5644insA, and the missense mutation p.Lys1981Arg. A missense mutation of Lys was not described in DEB association so far. The patient's phenotype associated with p.Lys1981Arg was milder in comparison with patients' phenotypes associated with substitutions of Gly and Arg detected in our DEB patients. **Conclusions:** In summary, screening the COL7A1 gene is useful for understanding different clinical variants of DEB.

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P12.064 Splicing abnormalities in the DMD gene

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The most common form of Duchenne and Becker muscular dystrophies causing mutations are large intragenic deletions and duplications that account for 60 to 80% of all cases. The remaining cases are caused by small mutations consisting of nonsense, missense, small insertion-deletions and a wide range of complex changes due to abnormalities of normal splice processing. Genomic based techniques have sometimes limited ability to detect such changes.

The technical approach that we used consisted in: 1) immunohistochemical and western-blot analysis of patient's muscle biopsies against dystrophin and different muscle dystrophy associated proteins, 2) exclusion of exonic deletion and duplication in DMD gene by MLPA technique, 3) muscle biopsy RNA sequencing of total coding region and, 4) further confirmation in targeted genomic DNA.

We describe one DMD and five BMD patients diagnosed by clinical and immunohistochemical criteria, in which further genetic analysis revealed the presence of different mutations that alter normal splicing process. Exceptions to the reading frame rule were observed in two patients.

Two of them destroy the normal donor-site, one leading to exon skipping and the other causing partial intron transcription. Another creates a new donor splice site inside the coding region deleting 5 exonic bp. The fourth one, creates a cryptic exon in deep intronic region. The fifth mutation located at the donor site, leads to normal splice efficiency reduction. In the last case, a BMD patient presents a nonsense mutation and a partial skipping of the mutated exon that could explain the milder phenotype that has been observed.

P12.065 Dyt1 mutations in primary torsion dystonia in Iranian patients

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Early-onset, generalized primary torsion dystonia (PTD) is an autosomal dominantly inherited disorder, characterized by involuntary movements and abnormal postures. The majority of cases are caused by a 3-bp deletion (GAG deletion at position 946) in the DYT1 gene on chromosome 9q34 that allows for specific genetic testing. Forty eight patients with early onset primary torsion dystonia were screened for mutation in exon 5 of the DYT1 gene using PCR and DNA sequencing.

ing. In this study, we examined 48 Iranian patients with dystonia, and found the mutation in 8 patients (17%). The results showed that the prevalence rate of DYT1 mutation in Iranian patients was not too much different from European (27.3%) and Asian (22.2%) patients with early onset primary torsion dystonia.

P12.066 Secondary genetic determinants of phenotypic severity in recessive dystrophic epidermolysis bullosa (RDEB)

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RDEB is a skin blistering disorder caused by type VII collagen deficiency, responsible for a wide spectrum of disease severity. Correlation between *COL7A1* genotypes and RDEB severity is incomplete, and we showed previously that an *MMP1* promoter polymorphism associates with higher severity. Using microarrays, we analyzed the transcriptome of cutaneous cells cultured from three brothers with consanguineous parents, homozygous for the same missense *COL7A1* mutation but respectively affected with localized, generalized, and severe generalized RDEB (RDEB^L, RDEB^G, RDEB^{SG}). RDEB^L fibroblasts expressed 10-fold higher levels of *TGFB2* mRNA than RDEB^{SG} cells (2.5-fold higher than RDEB^G), 2-fold higher concentrations of TGF-β2 in conditioned medium, and larger pools of SMAD proteins. Transcripts increased in RDEB^L fibroblasts relative to RDEB^{SG} clustered to gene ontologies of cell adhesion and extracellular matrix (ECM). NF-AT-binding motifs were overrepresented in the corresponding gene promoters. The reciprocal transcript set was heterogeneous and depleted in those gene ontologies. Interferon response motifs were remarkably overrepresented in the gene promoters. Transcripts increased in RDEB^L keratinocytes relative to RDEB^{SG} mapped to gene ontologies of mitosis, DNA replication and repair, and the cell cycle, with higher cyclin B mRNA levels. The reciprocal transcript set was depleted in those categories, and proliferation inhibitor *CDKN2A* mRNA was augmented. The fibroblast TGF-β2 profiles indicate a protective role for this pleiotropic cytokine as a regulator of ECM and several signaling pathways of immunity and inflammation. We observed no *TGFB2* allelic polymorphism in this RDEB sibship, however, suggesting that TGF-β2 changes are secondary to an upstream regulatory gene.

P12.067 Mutation R163W in the *KRT9* gene in a Mexican family with Epidermolytic Palmoplantar Keratoderma and new associated features

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Background: The epidermolytic palmoplantar keratoderma (EPPK), an autosomal dominant genodermatosis, is the most frequent form of the hereditary palmoplantar keratodermas. The EPPK is characterized by hyperkeratosis of the palms and soles. About 90% of patients present mutations in the *KRT9* gene, generally affect the highly conserved coil 1A region of the α-helical rod domain of keratin 9, a domain important for keratin heterodimerization. **Objective:** To perform a clinical and molecular study in a Mexican family with EPPK. **Methods:** We analyzed clinically and genetically a family with 12 affected members with EPPK. The *KRT9* gene was analyzed from genomic DNA through PCR and DNA sequencing analysis. **Results:** The 12 affected members of the family had hyperkeratosis of the palms and soles. We detect R163W mutation in the *KRT9* gene in all affected members of the family.

Conclusions: Although the R163W change in the *KRT9* gene is the most frequent mutation in EPPK, only 2 families have been reported with knuckle pads associated to this mutation.

P12.068 Congenital factor XIII deficiency caused by two mutations in eight Tunisian families: molecular confirmation of a founder effect

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Introduction: Inherited factor XIII (FXIII) deficiency is a rare bleeding disorder characterized by an umbilical bleeding during the neonatal period, delayed soft tissue bruising, mucosal bleeding spontaneous intracranial hemorrhage and soft tissue hemorrhages. Congenital FXIII deficiency is an autosomal recessive disorder, usually attributed to a defect in the FXIIIA and B subunits coding respectively by F13A and F13B genes. **Aim:** The aim of this study was to determine the molecular defects responsible for congenital factor XIII deficiency in eight Tunisian families. **Methods:** Molecular analysis was performed by direct DNA sequencing of polymerase chain reaction amplified fragments spanning the coding regions and splice junctions of the FXIIIA subunit gene (F13A) in probands and in families' members and compared with the reported sequence of this gene. **Results:** In all patients, FXIIIA activity was undetectable and the FXIIIB was within the normal range. Direct sequencing of the F13A gene in all probands showed two mutations: the c.869insC mutation found in eight patients and the c.1226G>A transition found in only one. We also confirmed the presence of a founder effect for the first frequent mutation by using two microsatellite markers, HUMF13A01 and a generated ployAC marker (HUMF13A02). **Conclusion:** We describe here molecular abnormalities found in nine Tunisian probands diagnosed with FXIIIA deficiency. The identification of the founder mutation and polymorphisms allowed a genetic counselling in relatives of these families and the antenatal diagnosis is now available.

P12.069 Frequency of *PRF1*, *STX11* and *UNC13D* mutations in patients with a genetic diagnosis of familial hemophagocytic lymphohistiocytosis

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Familial hemophagocytic lymphohistiocytosis (FHL) is a rare autosomal recessive lethal condition characterized by fever, cytopenia, hepatosplenomegaly and hemophagocytosis. It is usually rapidly fatal without adequate therapy. The hallmark of FHL is defect apoptosis triggering and deficient lymphocyte cellular cytotoxicity. Four disease-causing genes have been identified *PRF1*, *UNC13D*, *STX11* and, most recently, *STXBP2*. We have reviewed the frequency of biallelic mutations in different FHL genes in a large, multi-ethnic cohort of 54 patients/families with a genetic diagnosis of FHL. These patients were analysed up to mid 2009, no patients had then been analyzed for *STXBP2*. Biallelic mutations in *PRF1*, *UNC13D* and *STX11* were demonstrated in 27/54 (50%), 15/54 (28%) and 12/54 (22%) patients, respectively. We observed a significantly higher prevalence of *STX11* mutations in the Turkish patients compared to European patients (10/23 vs. 0/15, p = 0.003). In Middle East patients, *PRF1* was the most common disease causing gene (10/16, 63%).

Another autosomal recessive immunodeficiency associated with development of a hemophagocytic syndrome including partial albinism is Griscelli syndrome type 2 (GS2) caused by mutations in *RAB27A*. We have identified *RAB27A* mutations in five patients/families with a clinical diagnosis of GS2. In addition, we have identified two patients with X-linked lymphoproliferative syndrome (XLP) type 1 and one patient with XLP type 2 caused by mutations in *SH2D1A* and *XIAP*, respectively. Genetic analysis has proven to be a most helpful tool in diagnosis of FHL and related disorders. Moreover, genetics is essential for prenatal screening and carrier testing.

P12.070 Resequencing of *LDLR* and *APOB* genes in patients with clinical diagnosis of Familial Hypercholesterolaemia

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Familial hypercholesterolaemia (FH) is a monogenic condition caused in most cases by mutations in *LDLR* gene, but mutations in *APOB*

and PCSK9 genes are also cause of FH. These 3 genes are currently studied in the "Portuguese FH Study". From the 359 families with a clinical diagnosis of FH studied, only 48% of these have a detectable mutation in the 3 genes mentioned above so, other mutations in these 3 genes or other gene defects must exist to explain the cause of hypercholesterolemia in the remaining families. In order to find if a LDLR or APOB mutation was missed by current methods, the coding regions and exon/intron boundaries of LDLR and APOB gene, of 65 index patients with clinical diagnosis of FH and no detectable mutations in LDLR and APOB genes, were resequencing by a novel method, pyrosequencing. By this method a pool of the 65 DNAs was sequenced together several times and the results are given based on number of alleles estimated taking into account the frequency of each alteration, total number of reads obtained for each fragment and number of individuals sequenced. In the LDLR gene 41 alterations were detected, most of them are polymorphism, 3 were positive controls and 3 new alterations. In the APOB gene 87 alterations were detected, being 27 previously described SNPs.

Pyrosequencing allows the rapid sequencing of a large number of individuals, but apart from its high cost, it has some limitations and requires an improvement of technique.

P12.071 Portuguese Familial Hypercholesterolemia Study: comparison of the effect of LDLR gene mutations in FH patients

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Familial hypercholesterolemia (FH) is a common genetic disorder (1/500) associated with high levels of plasma cholesterol and premature coronary heart disease (pCHD). In the Portuguese FH Study the genetic diagnosis is based on molecular study of LDLR, APOB and PCSK9 genes, but mutations in the LDLR gene account for the majority of identified mutations. The aim of the present work was to analyse the phenotype of FH patients carrying a null or a defective allele mutation in *LDLR*.

To this date a total of 404 individuals were identified with a genetic defect in *LDLR*. Mean levels of total cholesterol and LDLc in the paediatric and adult group were calculated using SPSS v.17. Paediatric patients with null mutations presented mean total cholesterol of 312.88 ± 71.15 mg/dL and LDLc of 242.21 ± 67.74 mg/dL. Paediatric patients carrying a defective allele mutation presented levels of total cholesterol and LDLc of 272.15 ± 59.67 mg/dL and 196.75 ± 51.94 mg/dL. Adult FH patients with null mutations present mean total cholesterol of 369.33 ± 91.14 mg/dL and LDLc of 281.79 ± 99.62 mg/dL. Adult FH patients carrying a defective allele mutation presented a mean level of total cholesterol of 334.98 ± 67.94 mg/dL and LDLc of 250.19 ± 68.01 mg/dL. In the first group 21.4% had pCHD and in the second 13.3%. All differences observed were statistically significant.

Null allele mutations in *LDLR* gene (large deletions, small deletions/insertions, nonsense and splicing mutations) are predicted to result in the production of a non-functional protein and present this way a much severe phenotype. The type of mutation is important to access the cardiovascular risk of these patients.

P12.072 Study of LDL receptor gene mutations in patients with familial hypercholesterolemia in Chaharmahal va Bakhtiari province.

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Background and aim: Familial hypercholesterolemia is an autosomal dominant inherited disorder characterized by increased level of low-density lipoprotein cholesterol that leads to lipid accumulation in tendons and arteries, premature atherosclerosis and increased risk of coronary heart disease (CHD). Familial hypercholesterolemia is caused mainly by mutations in low-density lipoprotein receptor (LDLR) gene. The aim of this study was to analyse the LDLR gene mutations in a group of patients from chahar mahal va Bakhtiari province.

Methods: in this descriptive -lab based study ,57 suspected FH patients were screened for mutations in promoter and exons

1,3,5,11,13,15,16,17 and 18 of LDLR gene using PCR-SSCP strategy.

Results: Two different LDLR gene variations including heterozygote mutation 283T>A and polymorphism 1959T>C were identified in 1 and 9 FH Families studied respectively.

Conclusion: We conclude that LDLR gene mutation may not be the major cause of FH in the population studied and the cause of FH in chaharmahal va Bakhtiari province remains to be detected in other loci or genes.

Familial Hypercholesterolemia, Low density lipoprotein receptor gene,PCR-SSCP

P12.073 PCSK9 alterations in patients with Familial Hypercholesterolemia

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Familial hypercholesterolemia (FH) is characterized by increased levels of LDL cholesterol and premature Coronary Heart Disease (CHD). Although LDLR and APOB defects are more common causes of FH, mutations in PCSK9 also cause FH. The Portuguese FH Study was developed to identify FH patients in order to prevent the development of premature CHD. The aim of this study is the molecular analysis of PCSK9 gene, in patients with no mutation in LDLR or APOB gene. A total of 425 index patients have been studied and a mutation in the LDLR or in the APOB gene was found in 169 patients. Until now a total of 48 index patients without mutation in LDLR or APOB, 11 children (TC 256 ± 34.4 mg/dL; LDLc 189 ± 30.1 mg/dL) and 37 adults (TC 340 ± 43.6 mg/dL; LDLc 240 ± 36.9 mg/dL) were completely tested for genetic defects in PCSK9 gene. The 12 exons and exon-intronic boundaries of the PCSK9 gene were amplified by PCR and direct sequence. All possible mutations and polymorphisms were annotated. Two unrelated patients were found to be heterozygous for a novel mutation in PCSK9, predicted to cause a single amino acid substitution, D374H. Both presented a severe phenotype (premature CHD; patient 1, TC 567 mg/dL; LDLc 503 mg/dL; patient 2, TC 444 mg/dL). The polymorphisms, c.43_44insCTG in exon 1 and E670G, A>G variant in exon 12, showed a higher incidence in the Portuguese population than the revealed in other Caucasian populations. FH patients with PCSK9 mutation have a rare but more severe form of the disease.

P12.074 A novel mevalonate kinase gene mutation in combination with MVK V377I substitution and the common MEFV V726A mutation.

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Hyper-IgD (HIDS) syndrome is an autosomal recessive disorder characterized by recurrent episodes of fever associated with lymphadenopathy, arthralgia, gastrointestinal disturbance, and skin rash. HIDS is caused by mutations of MVK gene. Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by fever and synovial inflammation. FMF is caused by mutations affecting both alleles of gene MEFV.

In the present study we present data from a Sicilian patient with typical symptoms of the FMF, but only heterozygous mutation in MEFV (V726A) and compound heterozygous in MVK gene (V377I, P228L, inherited from the mother and father respectively). The P228L is a novel missense mutation involving the exchange of a proline (CCA), highly conserved in mammals and birds, with a Leucine (CTA).

The proband then is affected from unusually form of HIDS (IgD no detectable, proteinuria and serum amyloid A levels are of 120.00 ug/ml). This findings encourage our assumptions about the oligogenic transmission of the syndromes associated with periodic fevers, on the basis of the identification of 3 mutated alleles in 2 different genes. The third mutation in the gene MEFV could play a role epistatic, modifying the spectrum symptoms of HIDS.

Further study on HIDS and FMF wider population would be important not only to clarify the genetic heterogeneity of this family of syndromes, but mainly to establish a new diagnostic and therapeutic approach to these patients.

P12.075 MEFV gene mutations in patients with FMF from the middle Black Sea region of Turkey.

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Background: Familial Mediterranean Fever (FMF) is an autosomal recessive disease presenting with recurrent bouts of fever, inflammation of serosal membranes, skin rashes, and joint problems. FMF is frequently seen in populations of Arab, Armenian, Jewish, and Turkish ancestry. Disease is caused by mutations in MEFV gene, sequence variants of which totals 188 according to the Infevers Database. The purpose of the present study was to determine the frequencies of the 12 mutations in MEFV gene in patients with FMF from the middle Black Sea region of Turkey.

Method: We screened 3904 patients with suspicion of FMF for MEFV mutations using commercially available FMF StripAssay™ method, a multiplex PCR based reverse hybridization assay.

Results: Overall, MEFV mutations were detected in 47.80% of the patients, while no mutations were detected in 52.20%. Of the 2755 MEFV alleles identified, M694V allele was present at 46.75%; M680I 21.67%; E148Q 14.66%; V726A 8.78%; P369S 3.19%; A744S 1.38%; R761H 1.20%; F479L 1.13%; K695R 0.91%; and M694I 0.33%. Of the 1866 patients with MEFV gene mutation(s), 21.65% were homozygotes, 52.79% were heterozygotes with single mutations, 25.19% were compound heterozygotes with two mutations, and 0.38% were with complex genotypes (n: 7 patients; 2 patients with E148Q/E148Q/M680I/M680I genotypes, 2 patients with M694V/E148Q/P369S genotypes, 2 patients with M680I/E148Q/P369S genotypes, 1 patient with V726A/V726A/E148Q genotype).

Conclusions: We were able to identify 53 different allelic combinations of the 12 mutations tested in MEFV gene in Turkish patients in whom FMF was suspected. We will present, the genotype-phenotype correlations in depth.

P12.076 Detection of a hotspot for mutations in KITLG responsible for Familial Progressive Hyper- and Hypopigmentation**

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Familial Progressive Hyperpigmentation and Hypopigmentation (FPHH) is an autosomal dominant disorder with reduced penetrance. Clinical signs consist of progressive diffuse, partly blotchy hyperpigmented lesions, multiple café-au-lait spots intermingled with hypopigmented-appearing maculae, and lentiges. Histological and ultrastructural sections from the hyperpigmented lesions display strong basal hyperpigmentation of the epidermis with numerous melanophages containing large amounts of melanin. In contrast, the hypopigmented-appearing maculae show slight basal hyperpigmentation of the epidermis, with virtually no melanophages in the upper dermis. FPHH is distinct from FPH, in which no hypopigmented features are present, and which is phenotypically and histologically closer to Dyschromatosis Universalis Hereditaria 2 (DUH2). We performed a genome-wide linkage analysis in seven families with FPHH using Affymetrix SNP GeneChips, and identified linkage on 12q21.12-q22. This locus overlaps with that of DUH2. Moreover, one mutation was reported in the KITLG gene in this locus in a Chinese family with FPH. We discovered three different mutations in four of our FPHH families. The reported FPH substitution was found in two families, and two novel substitutions in the other two families. Thus, mutations in the same gene cause FPH and FPHH, and most likely DUH2. Interestingly, all the mutations are located in a highly conserved third β-strand in KITLG, suggesting an important role in activation of the downstream signalling pathway, which affects melanocyte migration and melanin distribution. This pathway groups other Mendelian disorders with dyspigmentation, such as piebaldism, neurofibromatosis type 1 and Legius syndrome. Moreover, hair and skin color was recently associated with the KITLG locus.

P12.077 Identification of novel loci linked to Familial Pulmonary Fibrosis

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Idiopathic Pulmonary Fibrosis (IPF) is pathologically characterized by inflammation and fibrosis of the lung parenchyma. It is a late onset disease with variable penetrance that generally develops from 50-70 years of age and is associated with an estimated survival of 20-50% at 5 years after diagnosis. The prevalence of IPF in Newfoundland is 117 cases per million, which is much greater than the prevalence in the UK of 1.34 cases per million. Familial Pulmonary Fibrosis (FPF) is pathologically indistinguishable from IPF; however an earlier age of onset is frequently observed in FPF families. *SFTPC*, *TERT*, *TERC*, *SFTPA2* and *ABCA3* are the genes known to be associated with the disease.

We hypothesize that there is a gene(s) carrying a mutation(s) that is the underlying cause of FPF in Newfoundland families. We sequenced the 5 known FPF genes in 59 probands and excluded them as FPF causing in the vast majority of individuals. Six families with 37 affected and 205 unaffected individuals were selected for novel gene discovery. The pedigrees are consistent with an autosomal dominant inheritance pattern, which is consistent with previous reports of FPF. Genome-wide scans with a 10cM microsatellite set and a 610k SNP-chip suggested linkage to multiple chromosomal loci.

Using parametric, non-parametric and homozygosity haplotyping analyses we identified a number of novel loci linked with FPF in these families. A candidate-gene approach is being utilized to select genes within the regions of interest for sequencing. Genes are selected based on association with lung diseases, function and mRNA expression.

P12.078 DNA bank for Polish patients with a predisposition for intestinal polyposis

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Intestinal polyposis syndromes include a group of diseases conditioned by the occurrence of hereditary mutations. Here we present a collection of DNA samples derived from persons from families with a diagnosed adenomatous polyposis which comprise: familial polyposis coli together with its recessive form, Turcot's syndrome, inherited mixed polyposis as well as persons with recognised hamartomatous polyposis: juvenile polyposis, Peutz-Jeghers syndrome, Cowden syndrome and Proteus syndrome. The objective of this study was to present current achievements associated with the establishment of the DNA Bank for intestinal polyposis. At the present time, the DNA 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. In addition, the collected data also contain material for analyses derived from 25 families with Peutz-Jeghers syndrome and 20 families with juvenile polyposis as well as single cases with the Cowden syndrome, Proteus syndrome and desmoid tumors. The performed molecular investigations allowed identification of mutations ranging from 44 to 50%.

The study was supported by the Polish Ministry of Science and Higher Education projects no. N402 481537, N401 331936

P12.079 STXBP2 mutations are not detected in group of Russian patients with Familial hemophagocytic lymphohistiocytosis (FHL).

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FHL is a rare inherited immune dysregulation syndrome characterized by a defect in natural killer cell function and caused by mutations in *PRF1*, *UNC13D* and *STX11* genes (FHL2, FHL3 and FHL4). These genes code the proteins involved in perforin-dependent NK and T cells cytotoxicity. A new genetic form FHL5 had been described in 2009. The causative FHL5 gene is *STXBP2* (19p) coding syntaxin-binding protein 2 (Munc 18-2). Munc 18-2 protein interacts with syntaxin 11 and is involved in the regulation of vesicle transport in NK cells and

cytotoxic T lymphocytes.

DNA samples of sixteen Russian FHL patients (3 girls and 13 boys) were examined for mutation in coding region of *STXBP2*. The diagnosis in all cases was established according to the criteria of the Histiocytic Society. The investigation of *PRF1*, *UNC13D* and *STX11* genes coding regions had not reveal mutations.

No mutations were detected in coding region of *STXBP2* gene in DNA samples of these patients. A number of previously reported SNP's were detected. Only a few new nucleotide substitutions were revealed:

c.247-29G>A (GL6, 7, 8, 25, 29, 30)
 c.429+12G>C (GL7, 8)
 c.794-4C>T (GL24)
 c.960+22C>T (GL25)
 c.902+32insC (GL29)
 c.1288C>T (p.430Arg>Cys) (GL7)
 c.1034C>T (p.345Thr>Met) (GL8)
 c.1375C>T (p.459Arg>Trp) (GL29)
 c.1538+35G>T (GL11)
 c.1697-34C>G (GL29)

Some of these polymorphisms could prove to be missense mutations, so the further population and functional analysis should be performed. FHL5 does not appear to represent a frequent form among Russian patients.

P12.080 Existence of FMF-like condition unrelated to MEFV

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Familial Mediterranean Fever (FMF) is the most prevalent hereditary autoinflammatory disease which arises from mutations of MEFV gene regulating the innate immune system. A systematic mutation screening among Armenians revealed a group of patients (n=46) with clinical symptoms of FMF but without demonstrable MEFV mutation compared to FMF patients with expected straight forward recessive form of inheritance. Given the frequent identification of single-mutation cases (30%) may be due to the recognition of a broader FMF phenotype, and extremely high frequencies of MEFV mutant alleles among healthy Armenian carriers (1:3), this supports the existence of new, FMF-like autoinflammatory condition unrelated to MEFV. The stratified genotype-phenotype analysis revealed some significant differences between two clinically-similar conditions. Although the cardinal feature of FMF, periodic fever was significantly higher among FMF patients ($p=0.0095$), still it was detected in 83% of FMF-like patients without MEFV mutations, which importantly explains nearly all cases of FMF in whom no MEFV mutation have been identified. Despite similar clinical picture, some features such as artralgia and skin elements were more frequent among patients with the suspected syndrome ($p=0.0001$). The search of autoinflammatory cytokine-markers also revealed a higher frequency and a higher level of IL-10 in FMF-like patients (0.77pg/ml in 33,3% cases) compared to healthy controls (0.41pg/ml in 16,3% cases) and FMF-patients (0.17pg/ml in 7,6% cases). Thus, changing the concept regarding broader FMF phenotype may call for a higher awareness of the existence of MEFV-unrelated autoinflammatory condition distinguishable from other autoinflammatory syndromes but yet indiscernible from MEFV-related FMF.

P12.081 Risk measure for expansion upon transmission in FMR1 grey alleles

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Fragile X Syndrome is due to an expansion of CGG-repeat tract in the 5'-untranslated region of the *Fmr-1* gene. In normal alleles, every 9-10 CGG repeats a trinucleotide AGG is inserted which seems to provide stability to this sequence. Its absence or deletion at the 3' end may be prone to expansion upon transmission.

Aim: To study AGG interspersion pattern in order to evaluate grey allele stability and its predisposition for fragile X syndrome.

Patients and Methods: Herein, we describe a study performed on 37 male blood samples among the 40-55 CGG repeats range coming from neuropediatric units and a group of 12 samples <40 CGG repeats as normal controls.

PCR test to amplify the CGG triplet repeat region of *Fmr-1* alleles was

performed followed by sequencing in order to identify the AGG interruptions.

To evaluate the instability we used an interspersion rate parameter. Statistics: Mann-Whitney test, ROC curve test and Spearman test and Box Plot to evaluate the diagnostic technical efficiency.

Results: After the 10 first CGG repeats, the number of interspersed AGG among grey alleles is nearly the half than among the normal control alleles this was evaluated with the Mann-Whitney test.

Values below or equal to a threshold value of 0.89 are considered instable alleles with high risk of expansion; with 91,7% sensitivity and 89,2% specificity.

Conclusion: Low "interspersion rate" is correlated with high risk of expansion upon transmission. We propose a revision of the instability risk of intermediate alleles, especially in genetic counselling for male-female transmissions.

P12.082 An unusual case of fragile X syndrome due to partial deletion of FMR1

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The most common cause of fragile X syndrome is the expansion of a CGG trinucleotide repeat in the 5'UTR region of FMR1, which leads to functional silencing of this gene by hypermethylation. However, in rare cases (< 1%) the loss of FMR1 gene function is due to partial or whole gene deletions, ranging in size from single nucleotides to several Mb. Here we report on a 9 year old boy with features compatible to fragile X syndrome. ArrayCGH using a whole genome 44k Oligo-array (Agilent) revealed a small deletion of 70-170kb on Xq27.3, including the gene FMR1NB and possibly a part of FMR1: arrXq27(146.844.547-146.915.743)x1 (ISCN 2009, hg18).

To further characterize this result and to determine if the deletion was maternally transmitted, we performed MS-MLPA for fragile X syndrome (Kit ME0289-B1, MRC Holland). This analysis confirmed a normal methylation pattern in the patient and his mother and a deletion of exons 15 to 17 of the FMR1 gene in the patient but not in his mother. Finally, the breakpoints were further analyzed by long distance inverse PCR (LDI-PCR) and sequencing.

Summarized, these results prove in our patient a de novo deletion of the 3' part of the FMR1 gene with the proximal deletion breakpoint in intron 14, protruding 118 kb distal including the FMR1NB gene. To our knowledge, this is the first case of a deletion of only the 3' part of FMR1 and the distally neighboring gene FMR1NB, resulting in a phenotype compatible to fragile X syndrome.

P12.083 Evidence for existence of a founder effect for the common mutation (W374X) in Perforin gene causing familial hemophagocytic lymphohistiocytosis

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Familial hemophagocytic lymphohistiocytosis (FHL) is a rare autosomal recessive disorder of immune dysfunction caused by mutations in Perforin, Munc13-4, Syntaxin 11 and recently *STXBP2* genes. Although FHL is a very rare disorder, it is relatively common in Turkish population probably because of the frequent consanguineous marriages. Perforin gene Trp374Stop (W374X) mutation in exon 3 (1122 G>A) appear to be the major genetic cause of FHL in Turkish population. In our studies on the molecular pathologies of FHL, we have identified this mutation in a total of unrelated 17 families coming from southeastern part of Turkey except 2 from middle part. Abreast studies of these families in haplotype analysis exploiting the genotyping of 5 different polymorphic microsatellite markers closely flanking Perforin gene indicated that W374X mutation was obviously segregated with the same conserved haplotype in all families except one where the mutation segregates not with the conserved haplotype but with only one of the conserved allele of a very close marker in linkage disequilibrium. The distribution of this allele was quite different between patients (1.0) and controls from healthy Turkish population (0.30). This and one family who has fully conserved haplotype were originated from the middle part of Turkey while all the rest 15 families from the southeastern part. These results may suggest that all families inherited the same disease allele from a common ancestor. In conclusion, this study provides evidence for the possible existence of a founder effect for the mutation. This study was supported by TUBITAK (Project No: 105S386-SBAG-3193).

P12.084 Molecular diagnosis for FMR1 related disorders

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Introduction: The Fragile X Mental Retardation 1 gene (*FMR1*) is associated with three distinct conditions: Fragile X Syndrome (FXS), Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) and Premature Ovarian Failure (POF). The most common molecular basis of *FMR1*-related disorders is an abnormal expansion of CGG repeats in the 5' untranslated region of the *FMR1* gene, which are hypermethylated, causing no *FMR1* expression. Based on the size of the expansion, it is possible to distinguish four types of alleles: normal (NL), intermediate, premutation (PM, associated with both FXTAS and POF) and Full-Mutation (FM, associated with FXS). Despite being a low-resolution and time consuming technique that requires large amounts of genomic DNA, Southern-Blot is still the most commonly used method for diagnosing *FMR1* related disorders. In alternative, we adopted the combination of direct PCR and fluorescent methylation-specific PCR (ms-PCR) (as published by Zhou Y. et al. 2006).

Method/ Results: From 300 samples studied by conventional PCR, 66 were referred to ms-PCR with the following results: 59 females had a normal ms-PCR pattern, 3 males with a FM pattern, 2 females and 1 male with a PM pattern and 1 female with a gray zone allele. All the results were verified by southern-blot confirming the sensibility of this method.

Conclusion: This approach confirms the efficiency to rapidly determine the allele status of males and females, including homozygous females on standard PCR, according to their unique GeneScan™ electropherogram patterns.

P12.085 Analysis of granulin gene in patients with familial frontotemporal dementia

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Frontotemporal lobar degeneration (FTLD; MIM # 600274) represents the second most common dementia subtype in patients younger than 65 years. Positive family history is observed in up to 50% of FTLD patients. Most of these cases are linked to a region in chromosome 17q21, and in some of these families the microtubule-associated protein tau gene (MAPT; MIM # 157140) was found to be the causative gene. Other families with FTLD mapping to 17q21 and without variations in MAPT, carried mutations in the gene encoding progranulin and known as granulin (GRN; MIM # 138945).

Mutation screening of GRN gene was performed in 11 unrelated patients with familial FTLD using automatic sequencing. We did not identify any pathogenic mutation in coding regions or intronic boundaries. However, five different polymorphisms were found (one not reported in the literature) and the identification of a risk haplotype combining these polymorphisms and others already described is being carried out.

P12.086 Segregation of a new mutation in SLC26A4 and E47X mutation in GJB2 within a consanguineous Tunisian family affected with hereditary deafness

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Autosomal recessive forms of hearing loss account for approximately 80% of genetic cases. At least 60 genes have been identified to cause autosomal recessive syndromic and non-syndromic hearing loss. Among these genes, *SLC26A4* in which mutations have been reported to be responsible for non syndromic hearing loss (DFNB4) and Pendred syndrome characterized by the association of sensorineural hearing loss and the presence of goiter. This gene encoded a chloride transporter protein called Pendrin. Mutations in *GJB2* gene result in autosomal recessive (DFNB1) and dominant (DFNA3) non-syndromic hearing loss. We describe a Tunisian Consanguineous Family showing linkage to *GJB2* and *SLC26A4* genes. Mutation analysis of these

two genes revealed a novel frameshift mutation [c.451-delG] and the E47X mutation in the same family. Haplotype analysis for microsatellite markers and single nucleotide polymorphisms (SNPs) closely flanking the *GJB2* gene revealed the presence of two haplotypes associated with the E47X mutation, suggesting that two founder effects for this mutation are responsible for hearing loss among Tunisian population. This report presents an illustration of how consanguinity and founder effect could increase familial clustering of hereditary mutations within the same family.

P12.087 MLPA technique identifies large deletions in Gitelman syndrome

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Gitelman syndrome (GS) is part of the renal tubulopathies, characterised by hypokalemia, metabolic alkalosis, high renin and aldosterone. The disease is inherited as an autosomal recessive trait.

Most mutations detected in GS are point mutations defined by sequencing. About 30% of GS-patients show only one mutation after coding sequences analysis.

Rarely, deletions of one or more exons have been described in GS-patients. We therefore hypothesized that the missing mutation of our heterozygous GS-patients could be a deletion or a duplication (del/dup). We screened 13 heterozygotes for abnormal exon copy number, plus two brothers showing a homozygous absence of PCR product in exon 26.

The Multiplex Ligation-dependent Probe Amplification was performed using the commercial kit (MLPA®, MRC Holland) to determine the copy number of 25 out of the 26 exons of the *SLC12A3* gene.

The MLPA fragments were loaded on 3130xl Genetic Analyzer (Applied Biosystems) and analysed using the appropriate software.

MLPA confirmed the homozygous deletion of exon 26 in the 2 brothers; four heterozygotes showed a 50% copy number reduction of at least one exon.

Direct sequencing permits a high detection rate for point mutations whereas large del/dup are missed.

We have introduced the MLPA technique as second screening after sequencing the coding region of the gene. It will be interesting to see the proportion of large del/dups among mutations in GS and their impact on the disease. This will furthermore permit to define if the proportion of del/dup could justify the introduction of MLPA as a first screening method.

P12.088 Phenotypic variability of non-syndromic hearing loss in a Lur family due to delE120 mutation in GJB2 gene

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Introduction: Hearing loss is the most common sensory defect in the world. The genetic basis of this condition is very complex. Molecular variations in *GJB2* gene are the common cause of hearing impairment in Caucasians. One expects that affected members of a family with same mutation have similar phenotype. Here we report phenotypic variability in hearing loss among the members of a Lur family.

Case Presentation and Methods: A Lur family from Lorestan province in western Iran having hearing impairment came for genetic counseling. There were two brothers with variable degrees of nonsyndromic sensorineural hearing loss. Clinical examinations, audiological tests and molecular studies including *GJB2* gene sequencing and detection of Δ (GJB6-D13S1830) deletion were performed.

Results: Sequencing *GJB2* gene revealed delE120 mutation in both brothers in homozygote form. Since one of them was profoundly deaf and the other was mildly affected, we were expecting different genotypes or other causative effects. Δ (GJB6-D13S1830) not was found.

Discussion: Phenotypic variability between members of different family members with the same type of mutation can be expected which may

be due to the role of modifying factors but within the same family is less likely, particularly to the extend seen in our study.

P12.089 The inheritance of a missense c.487A>G mutation in GJB2 gene in two Iranian families.

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Background: Mutations in GJB2 gene are the most common cause of hereditary hearing loss. The majority of GJB2 mutations are recessive, but a few dominant mutations have been associated with hearing loss. This study introduces some new fact about M163V mutation in GJB2 gene in two Iranian families.

Material and Methods: Genomic DNA of Two unrelated Iranian families with sensorineural hearing loss were obtained from six family members and screened for GJB2 mutations with direct sequencing.

Results: Fathers of both families showed late onset hearing impairment in fourth decade of the life, but hearing loss in children was early onset in both families with more severity rather than fathers. Also, one grandfather of every family showed late onset hearing loss in seven decade. The analysis of familial pedigree revealed anticipation in phenotype and autosomal dominant inheritance. There was a substitution of A to G in exon 2 at nucleotide 487(M163V). This mutation was heterozygous in fathers and children while mothers were normal.

Discussion: Previously, M163V always introduced as unknown heterozygous not even as compound heterozygous. Researcher showed the produced protein of M163V failed in the formation of homotypic junctional channel.

Due to other mutation in this nucleotide was reported as M163L in autosomal dominant inheritance that defects trafficking to the plasma membrane and increase cell death.

Our finding can confirm the autosomal dominant inheritance of this mutation. This hypothesis was further supported by conservation of the methionine residue at position 163 across the 23 mammalian species.

P12.090 Molecular analysis of the GJB2 gene in congenital sensorineural hearing impairment in a sample of Mexican patients

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Congenital sensorineural hearing impairment is the most common sensory defect with an incidence of approximately 1 in 500 newborns. Almost half of cases of hearing loss are due to genetic factors of which 70% are classified as nonsyndromic. Autosomal recessive transmission remains the most frequent. The discovery of different mutations leading to hearing loss has led to clarify the molecular basis of disease. Mutations in the GJB2 gene, which encodes for connexin 26, is responsible for more than half of cases of genetic origin. In the present study we analyzed the GJB2 gene in 16 Mexican families and found in 87% of cases different mutations. A relevant fact was the high prevalence of heterozygous, we consider that the most likely cause of hearing loss was additional participation of the GJB6 gene, at least in the analyzed sample.

P12.091 Study of two deletions in GJB6 gene as the second mutant allele in GJB2 heterozygous autosomal recessive non-syndromic hearing loss subjects in Iran.

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Hereditary non-syndromic hearing loss is inherited in autosomal recessive pattern in about 80% of cases. Mutations in GJB2 gene (connexin 26) and two deletions in the GJB6 gene (connexin 30); del (GJB6-D13S1830) and del (GJB6-D13S1854), are accounted for 50% of au-

tosomal recessive hearing losses in some regions. Approximately 10 to 50% of the patients with GJB2 mutations carry only one mutant allele. This study aims to determine whether GJB6 deletions are the second mutant allele causing the disease in the GJB2 heterozygous cases studied.

We examined 45 unrelated GJB2 heterozygous autosomal recessive non-syndromic hearing loss subjects for presence of del (GJB6-D13S1830) and del (GJB6-D13S1854) mutations, using multiplex Polymerase Chain Reaction.

We detected none of the two deletions of GJB6 in the patients studied. So GJB2 gene deletions; GJB6-D13S1830 and GJB6-D13S1854 are not the second mutant allele in patients with only one GJB2 mutant allele in studied samples in Iran.

P12.092 Compound heterozygosity for Hemoglobin Knossos and cd 39 mutation in a young Romanian patient

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In the frame of beta-thalassemia mutation screening study, we investigated the first Romanian patient with thalassemia major due to compound heterozygosity for Hb Knossos and cd 39 (C-T) mutation. Hb Knossos (cd 27 [G-T]) is characterized by reduced synthesis and by interaction with beta-thalassemia, in which the double heterozygotes display typical features of thalassemia intermedia. Here we report the first case of Hb Knossos in our country.

Molecular analysis of the mutations in the β -globin gene has been performed using the PCR based methods: DGGE, ARMS-PCR and PCR-RFLP. Direct DNA sequencing confirmed that the propositus is compound heterozygous for Hb Knossos (cd 27 GCC-TCC) and cd 39 (C-T) mutation.

Hb Knossos is a variant with a single base substitution causing amino acid replacement and alternative splicing of precursor beta-messenger RNA by activating cryptic donor sites in the exon I. CAG-TAG substitution at codon 39 in beta-globin gene changes codon 39 into a stop codon terminating translation.

Hb Knossos displays a slightly decreased oxygen affinity; this factor may compensate in part for the severe anemia of the double heterozygotes. In our case co-inheritance of Hb Knossos with severe β^0 mutation causes the beta-thalassemia major phenotype and this is important for genetic counseling. *This work was supported by the grant PN II 41-045 from the Romanian Ministry Education and Research.*

P12.093 Screening of genes causing Huntington disease Like phenotype

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Huntington disease (HD) is an autosomal dominant disorder of the central nervous system, characterized by involuntary choreic movements, progressive motor impairment, psychiatric symptoms and cognitive decline. HD is caused by an expansion of CAG trinucleotide repeats in the IT15 gene located on chromosome 4p16.3, encoding for Huntingtin. There is a group of disorders with clinical features of HD but negative for trinucleotide repeats expansion in the IT15 gene, known as HD-Like disease: Huntington disease like 1 (HDL1), an autosomal dominant disease, caused by an extra octapeptide repeat in the prion protein gene (PRNP), on chromosome 20p12; HDL2, with autosomal dominant inheritance, caused by CAG repeats expansion above 40 repeat in the Junctophilin-3 (JPH3) gene, on chromosome 16q24.3. Furthermore, two dominant spinocerebellar ataxias (SCAs), dentatorubralpallidoluysian atrophy (DRPLA) and Spinocerebellar Ataxia (SCA17), may also have overlapping symptoms with HD.

We report a group of 455 Italian patients with Huntington phenotype: 154 out of 455 resulted negative for the IT15 CAG expansion. These patients were firstly tested for JPH3 gene CAG expansion, but we did not find CAG expansion in any patient, according to the previous

data in Caucasian population. Then, we tested the patients for other HD-like genes: PRPN gene, Atrophin 1 (ATP1) gene and TATA-binding protein gene (TBP). All patients resulted negative for all tested genes excluding one patient carrying TBP expansion causing SCA17. Our study confirms the importance of testing all actually known genes involved in HD-like phenotypes.

P12.094 The most common causes of hearing loss in patients Referred to genetic counseling.

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Objectives: Hearing impairment is a common disorder that affects about 10% of general population and its prevalence increases with age. One in 1000 children is born deaf, with about 60% of case being due to genetic factors in developed countries. Mutations in the Connexin 26 (GJB2) and Connexin 30 (GJB6) genes cause nonsyndromic deafness. Mutations in the Connexin 26 gene play a major role and account for about 50% of all autosomal recessive deafness in Europe. The most common mutation in northern Europeans is the deletion 35delG (GJB2). GJB2 mutation, 167 delT occurs almost exclusively in the Ashkenazi Jewish.

Patients and methods: The aim of the study was to elucidate the causes of hereditary nonsyndromic loss of hearing in 45 members of unrelated families with the diagnosis of grade III-IV nonsyndromic bilateral sensorineural deafness undergoing genetic counseling. The search for mutations in the GJB2 (35delG, 167 delT) gene and GJB6 (delGJB6-D13S1830) gene was performed.

Results and Conclusions: We have detected mutations in 18 out of 45 persons screened. Eleven persons were homozygous, six heterozygous for 35delG mutation, one had compound heterozygosity for 167 delT/ delGJB6-D13S1830, and one had heterozygosity for delGJB6-D13S1830 mutation. It was possible to consider the reason of decrease in hearing established at 11 patients on which both chromosomes it has been revealed 35delG, and at one patient with compound heterozygosity for 167 delT/ delGJB6-D13S1830. The mutations in Connexin genes are the common reason of hearing loss in Belarus.

P12.095 Mutation screening of mitochondrial DNA in non-syndromic hearing loss

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Background: Maternally inherited deafness accounts for approximately 1% of hereditary hearing loss (HL). Several mitochondrial mutations have been associated to this condition, but their occurrence and penetrance among different populations remain poorly investigated. Aim of our study was to screen mitochondrial DNA in Italian patients affected by non-syndromic sensorineural HL. 252 unrelated patients and 26 normally hearing subjects have been screened for the mitochondrial mutations most frequently reported associated to deafness (A827G, 961delTinsC(n), C1494T, A1555G, A3243G, A7445G, 7472insC, T7510C and T7511C), by using dHPLC and sequencing. All samples have been also checked for mutations in the connexin genes GJB2/ GJB6.

Results: A1555G, A3243G, A827G, 961delTinsC(n) mutations have been found each in 1/252 (0.4%) patients. The last two mutations were also detected each in 1/26 (3.8%) normal hearing subjects. No one of the other common HH-associated mutations has been detected. In addition, four new variants have been found in four patients: 3168 insT, A3261G, 7471delC and A7576G.

For A3261G and 7471delC variants the analysis of the tRNA secondary structure as well as the check of phylogenetic conservation strongly suggest a pathogenetic role.

Conclusion: The mitochondrial mutations commonly associated to HH resulted less frequent among the Italian patients than in other populations. A827G and 961delTinsC(n) variants could be either very low penetrance mutations or non-causative polymorphisms, whereas two novel variants here detected are suggestive for having a causative role in HH.

P12.096 New mutations in *WFS1* gene are involved in isolated deafness with ascending or U-shape audiometric curves, as well as "frontier phenotypes"

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Introduction: Wolfram syndrome (also called DIDMOAD for Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy and Deafness) is an autosomal recessive syndromic disease due to mutations in *WFS1*. Dominant mutations of *WFS1*, usually located in exon 8 and affecting C-terminal region of the protein wolframin, are also found in non-syndromic deafness, predominantly affecting low frequencies (DFNA6/14/38).

Methods: We have screened *WFS1* gene in a wide cohort of sporadic or familial cases of non-syndromic deafness, characterised by ascending or U-shape audiometric curves.

Results:

Fifteen heterozygous mutations (including eleven missense mutations and one duplication) have been identified in *WFS1* sequence, some of them being present in several unrelated families. We could also detect *WFS1* mutations in « frontier phenotypes », meaning autosomal dominant deafness associated with either diabetes mellitus or optic atrophy.

Conclusion: The molecular screening of *WFS1* is recommended in non-syndromic hearing impairment predominantly affecting low or middle frequencies. It might also be useful in autosomal dominant « frontier phenotypes » associating deafness and either optic atrophy or diabetes.

P12.097 Genotype phenotype correlation in Iranian patients with Hemoglobin H disease

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Background: Some genotypes of HbH patients have severe anemia and are dependent on regular blood transfusion. This retrospective study provides the molecular genetics of HbH disease and determines the genotype-phenotype correlation in a group of HbH patients in Iran.

Methods: DNA analysis of 40 patients with HbH disease was performed by Polymerase Chain Reaction. Suspected cases with α-thalassemia was more analyzed by Reverse dot blot hybridization and sequencing to confirm the diagnosis.

Results: Of the total 40 respondents, 27 (67.5%) were female and 13 (32.5%) were male with mean age 25.73 (SD±16.07) years. Eight patients (20%) were undergoing regular blood transfusion from whom four (10%) needed blood transfusion after 30 years of age. Five (12.5%) underwent irregular transfusion, and 27(67.5%) didn't have history of any transfusion. Four patients (10%) had a history of splenomegaly, and severe hypochromic microcytic anemia.

Most frequent mutations observed, were $\alpha^{3.7}/\sim\text{MED}$ in 10 cases (22.5%) and $\sim\text{20.5}/\sim\text{5 nt}\alpha$ in 6 patients (15%) and $\sim\text{20.5}/\sim\alpha^{3.7}$ in 4 cases (10%). We could find a positive correlation between mutation and transfusion dependency (P value =0.03)

Conclusion: Regarding to results we can conclude that particular genotypes of alpha thalassemia may produce mild disease with no need for transfusion and are not recommended for prenatal diagnosis. On the other hand some special genotypes such as $\sim\text{MED}/\alpha^{CS}\alpha$, $\alpha^{CS}\alpha/\sim\alpha^{CS}\alpha$, and $\alpha^{\text{PolyA1}}\alpha/\alpha^{\text{PolyA1}}\alpha$ lead to regular or irregular transfusion dependency. In this condition prenatal diagnosis is necessary and may advise parents for abortion.

P12.098 Rapid, sensitive and discriminatory HbS and HbC mutation detection using High Resolution Melting Analysis

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Background: Hemoglobinopathies are common monogenic diseases forming a major public health problem due to their severity and disabling nature. Genetic identification of the two most frequently ob-

served missense mutations in the beta-globin gene, HbS and HbC, are important epidemiologically and aid in prevention of the sickle cell trait and other serious hemoglobin disorders.

Aim: Our increasing patient population of Mediterranean, African and Middle Eastern origin urge for the need of a rapid, inexpensive and high-throughput genetic testing for HbS and HbC variants.

Results: Classical high resolution melting analysis with an optimized PCR amplicon of 110 bp allowed a sensitive and reliable identification of the two neighbouring HbS (c.20A>T) and HbC (c.19G>A) mutations. Discriminatory melting profiles were observed for all possible combinations of mutations: HbAA, HbAS, HbAC, HbSS, HbCC and HbSC, and confirmed the results obtained by Hb-chromatography and PCR followed by restriction digestion. Within the wild type control population tested, mainly consisting of Belgian and North African individuals, two other aberrant melting patterns were observed. Sequencing of all samples with aberrant melting patterns revealed one polymorphism at position c.9 either in heterozygous or homozygous state. Further testing including samples from over 35 other nationalities did not disclose other melting profiles.

Conclusion: This HbS and HbC HRM seems a promising, inexpensive and high-throughput alternative to PCR and restriction digestion analysis, although further validation is needed prior to implementation in a post- and prenatal diagnostic setting.

P12.099 Molecular analyses of F8 gene in Serbian Hemophilia A patients

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Hemophilia A is an X-linked recessive bleeding disorder. It is caused by mutations within F8 gene, which result in deficient activity of the factor VIII. More than 900 various mutations of F8 gene have been detected and reported in HAMSTeRS database, until now. In order to initiate characterization of mutations in Serbian hemophilia A patients, we started with detection of the most frequent rearrangements of F8 gene, inversion of intron 22 (type 1 and type 2) and intron 1. Using inverse shifting-polymerase chain reaction (IS-PCR) we screened 24 patients and their families, so far. Inversion of intron 22 was found in 6 patients (25%), Inv22-type 1 in 5 patients and Inv22-type 2 in one among them. Analyses showed presence of intron 1 inversion in 2 patients. All identified mutations were associated with severe phenotype. Carrier status was also analyzed in overall number of families. All of these results will be further discussed and compared with the present literature data.

P12.100 An inverse shifting-polymerase chain reaction for genotyping hemophilia-Causative rearrangements involving *int22h* and *int1h* hotspots in the factor VIII

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Hemophilia A (HA) is an X-linked coagulation disorder with a worldwide incidence of approximately 1 in every 5000 males. Almost one half of patients with severe HA have large inversions that disrupt either intron 22 (Inv22) or intron 1 (Inv1) of the factor VIII gene (F8). In order to improve the molecular diagnosis of Inv22 and Inv1, due to problems in using southern blotting and Long-PCR strategies for detection of Inv22, efforts are being made to generate new diagnostic tests. Recently, inverse shifting-PCR (IS-PCR) has been developed to overcome this defect. Here we report the reproducibility of the modified method in genotyping of Inv22 and Inv1 in some Iranian HA patients. For this purpose, 4 known cases (2 affected males with Inv22 and 2 carrier females) previously confirmed by southern blot and 7 cases (3 severe HA males, 2 carrier females and 2 normal males) were analyzed. *Bcl1* digestion followed by self-ligation to create *Bcl1 circles*, and finally PCR was performed on genomic DNA. A perfect match was obtained for 4 cases analyzed by southern blot. In new patients, one affected male and one carrier female were positive for Inv22. It seems IS-PCR technique have proven to be a rapid, robust and reliable technique for genotyping of inv22 in HA patients.

P12.101 WKN1/HSN2 gene analysis in two families with hereditary sensory and autonomic neuropathy type II

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Hereditary sensory and autonomic neuropathies (HSANs) are clinically and genetically heterogeneous. They are classified into five different types characterized by variable sensory and autonomic dysfunction due to peripheral nerve degeneration. Currently at least seven genes are associated with HSANs. Mutations in the protein kinase with-nolysine(K)-1/hereditary sensory neuropathy type 2 (*WNK1/HSN2*) gene cause autosomal recessive HSAN type II, an early-onset ulcero-mutilating sensory neuropathy. *HSN2* is a nervous system-specific gene within the *WNK1* gene which is located on 12p33.33. In the present study we report two unrelated Mexican families with *HSN2* due to *HSN2* gene mutations. The only exon of *HSN2* was PCR amplified and sequenced from genomic DNA. Diagnosis of *HSN2* was made based on clinical findings. We identified a mutation in the coding region of *HSN2*. Both families harbour a homozygous deletion of eight nucleotides that predicted to cause a novel stop codon located 11 nucleotides after the original stop codon. Such mutation potentially creates a protein with additional amino acids that may disrupt any regulatory function of the 3' untranslated region downstream from the normal stop codon.

P12.102 Identification of a new locus on chromosome 9p for a syndrome associating Spastic Paraplegia, Axonal Neuropathy and congenital Cataract segregating in an Autosomal Recessive pattern (SPANCATAR syndrome)

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Hereditary Spastic Paraplegias (HSP) as well as Axonal Peripheral Neuropathies (APN) could be transmitted as dominant or recessive traits. HSP are subdivided in pure and complicated affections. They are clinically characterized by progressive bilateral lower-extremity spastic weakness due to corticospinal tract deficits. Degeneration is maximal at the distal ends of the corticospinal tracts and, to a lesser extent, at the distal ends of dorsal column fibers. Both axonal and demyelinating peripheral neuropathies are also known as Charcot Marie Tooth disease that is the most frequent neuropathy with an incidence of 1/2500.

Many years ago, in 2 large consanguineous families originated from north Africa, including 2 and 3 affected individuals respectively, we performed a wide genome search using 400 microsatellites markers. We identified positive Lod Scores in different regions of the genome. Additional polymorphic markers were necessary to test these regions. All of them were excluded except one on chromosome 9 P. More than 20 additional microsatellite markers were tested in this last region leading to a 30 cM homozygous interval of segregation in the 2 analyzed families. Other families with the same phenotype are recruited in order to refine the locus and identified the responsible gene.

P12.103 Genetic analysis of the high bone mass phenotype segregating in a small Spanish family

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High bone mass (HBM) was originally defined as an asymptomatic autosomal dominant condition characterized by increased bone mineral density (BMD) due to gain-of-function mutations in the *LRP5* gene. In the general population, BMD is normally distributed, and at the high extreme of the curve people display BMD values similar to those found in HBM patients. The range of densities of HBM is defined by a sum Zscore > 4 (totalLS-Zscore + TotalHip-Zscore). In the BARCOS cohort of postmenopausal women, 0.6% of individuals display BMD values in this range (10 probands). Whether they have mutations in the *LRP5*

gene is unknown. We present one familiar case (family of proband n°10) in which the mother and one of the two sibs have BMD values in the range of HBM: mother, sum Zscore = 5.12; HBM-like son = 5.01, normal son = 3.41.

As a first approach, we undertook sequencing of relevant exons (2, 3, 4, 9, 10, 11, and 12) of the *LRP5* gene, plus all the coding sequences of the *DKK1* gene, and no mutations were found.

A second approach consisted on cosegregation analyses of markers within several candidate genes and this phenotype. Combining SNP-Plex genotyping and results from a SNP array we could exclude the following genes: *LRP5*, *DKK2*, *IL6R*, *RANK*, *BMP2*, and *KRM1*. The only gene cosegregating was *RANKL*. As a final step, we sequenced *RANKL* in the proband but no mutations were found. Thus, *LRP5* is not the cause of HBM in this family, neither are several other candidates.

P12.104 Genotype-phenotype correlation in families with myelin protein zero (*MPZ*) gene mutations

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Hereditary motor and sensory neuropathy (HMSN) is the most common cause of inherited peripheral neuropathies with a frequency estimated at 1/2500. Electroneuromyographic examination distinguishes a demyelinating forms (CMT1) and an axonal forms of the disease (CMT2). The *MPZ* gene encoding basic protein of the peripheral myelin. Different HMSN forms are resulted *MPZ* gene mutations.

Electrophysiologically, pathologically and genetically examinations were performed to Russia CMT patients. We evaluated demyelinating and axonal features of 36 patients from 17 families with *MPZ* mutations. The demyelination polyneuropathy with early onset and lower motor conduction velocity (MCV) in median nerve was detected in 15 families (88%). The axonal polyneuropathy with late onset and normal MCV was detected in two families (12%).

Mutation's allocation in protein's domains were analyzed by us. In the majority of cases (81%) mutations localize in Ig-like domain. However, correlation between clinical implications and mutation's location were not found in comparably with other author.

P12.105 Novel Rearrangements in Partial Deletions In The *PMP22* Gene in Two Spanish Families

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Hereditary neuropathy with liability to pressure palsies (HNPP) is an autosomal dominant inherited disorder characterized by episodic and recurrent demyelinising neuropathy of the peripheral nervous system. HNPP is mainly caused by a 1.4 Mb deletion in the CMT1A/HNPP region at 17p11.2-12, which includes the *Peripheral Myelin Protein 22* (*PMP22*) gene. This region has an elevated rate of rearrangements because of a high density of repetitive elements, which account for 43.37% of the entire CMT1A/HNPP region. Alu sequences represent around 23% of these repetitive elements.

We studied the 1.4Mb deletion in two families with HNPP, using allelotyping studies and MLPA. Only with the MLPA kit we were able to identify two partial *PMP22* gene deletions. For further characterisation of these deletions we performed a qGenomics array:

*Family CMT-482: The deletion spans about 30.46 kb, from exon 8 of *CDRT1* gene to exon 4 of *PMP22* gene. Both breakpoints occur in a 36 pb region of perfect homology, within two Alu sequences, located in *CDRT1* intron 7 and *PMP22* intron 4.

*Family CMT-1678: The deletion spans about 368 kb, from exon 3 to 3'UTR of the *PMP22* gene. The rearrangement is probably enhanced by the presence of two shared bases (AG) at the breakpoints that are located in intron 2 and 3' UTR of *PMP22* gene.

These results show that MLPA improves the sensitivity of the genetic diagnosis of HNPP and contributes to understanding the molecular mechanisms of genomic rearrangements in the CMT1A/HNPP region.

P12.106 Application of SNP-arrays to the identification of genomic rearrangements causing inherited metabolic disease

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Our group performs the genetic diagnosis of more than 50 different inherited metabolic diseases (IMD). In all the IMD studied there is a variable number of patients with incomplete genotype after application of standard mutation detection techniques (sequencing of PCR-amplified cDNA and/or exonic fragments) which do not allow the identification of pathological genomic rearrangements (deletions, duplications...). We have applied array-based technologies to detect this type of genetic lesions in patients with IMD. We have used the Illumina platform for whole genome analysis (Human Hap-Quad 610K) and DNA extracted from blood or fibroblast samples. We have identified novel deletions affecting the *ALDH7A1* gene (causing piridoxine-dependent epilepsy, OMIM 107323), the *GLDC* gene (causing non-ketotic hyperglycinemia, OMIM 239300) and the *BCKDHA* gene (causing maple syrup urine disease, OMIM 608348). In one patient with homocystinuria, *cbfE* type (OMIM 602568) and in one propionic acidemia patient (OMIM 606054), SNP-arrays revealed the presence of copy neutral homozygous segments encompassing the corresponding genes, consistent with segmental uniparental disomy (UPD) being the underlying mechanism of disease. These are the first cases of UPD causing homozygosity for a pathogenic mutation in these diseases. Further microsatellite analysis will determine the pattern of parental segregation of the corresponding chromosomes giving clues to the mechanism leading to UPD. The results underscore the importance of completing the genetic analysis using novel technologies and of testing the parents of a child with an autosomal recessive disease to provide accurate genetic counselling especially in terms of recurrence risk for future pregnancies.

P12.107 Clinical and genetic study of an Italian family with Autosomal Recessive Spastic Paraplegia associated with dysarthria and hearing loss

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Hereditary spastic paraplegias (HSPs) are genetically and phenotypically heterogeneous disorders characterized by progressive spasticity in the lower limbs. Both "uncomplicated" and "complicated" forms have been described. HSPs may be inherited as an autosomal dominant (AD), autosomal recessive (AR), or X-linked form. To date, 19 AR-HSPs loci have been mapped. The AR-HSPs have varying age at onsets and are mostly complicated forms. The aim of this study was to perform a linkage analysis in a small consanguineous AR-HSP Italian family comprising five members one unaffected and two affected siblings in which the parents were first cousins. The neurological examination of the two affected members revealed spastic paraplegia, cerebellar dysarthria, hearing loss, Babinski and Hoffmann signs. Age at onset was in childhood. Autozogosity mapping was performed using microsatellite markers linked to the following AR-HSP loci: SPG5, SPG7, SPG11, SPG14, SPG15, SPG20, SPG21, SPG23, SPG24, SPG26, SPG27, SPG28, SPG30, HSP-TCC and to three recessive spastic-ataxia loci (SACS, SAX3, SAX2). In the two affected patients a homozygous region was observed only with the microsatellite markers associated to SPG26 locus on chromosome 12. The haplotype reconstruction and analysis of recombination narrowed the SPG26 locus to a 20 cM region flanked by D12S59 and D12S1649 markers. As the current SPG26 locus on chromosome 12 is still large to select candidate genes, analysis of additional SPG26-linked families could be useful to further refine the candidate region and facilitate the selection of candidate genes.

P12.108 Juvenile Huntington disease in Russian familiesG. E. Rudenskaya¹, N. M. Galeeva¹, S. A. Kurbatov²;¹Medical Genetics Research Center, Moscow, Russian Federation, ²Genetic Counseling Department, Voronezh, Russian Federation.

Juvenile Huntington disease (JHD) manifests before 21 years and amounts 2-9 per cent of all HD cases. JHD pathogenesis is related to anticipation and imprinting, its typical features are akinesia and rigidity (in contrast to chorea in adult HD), paternal inheritance and huntingtin mutations with amount of CAG repeats >60, though atypical cases exist and maternal inheritance is possible. Eight JHD cases, 6 unrelated and 2 brothers, are presented. Seven patients, one of the brothers among them, had rigid JHD with onset in 7-12 years and mutations containing 63-81 repeats; in the second brother (57 repeats) JHD started in 20 years and represented like hyperkinetic form with no dementia. All cases were familial, but HD history in some was not evident due to anticipation and/or erroneous diagnosis. Thus, HD in a grandfather started in 60 years, 7 years after JHD onset in his granddaughter (78 repeats); JHD in the mother started in 19 years and was misdiagnosed as multiple sclerosis. Maternal JHD transmission was seen in other two families, both mothers had "classic" HD with relatively early onset. One more family illustrates anticipation and differences between sibs: JHD in a girl started in 10 years (78 repeats), her 19-year-old brother was asymptomatic (49 repeats), HD in the father started in 48 years (45 repeats). In many cases diagnosis was delayed (up to 18 years after onset) which proves JHD underestimation. JHD should be considered even in 'non-familial' cases, and DNA testing for huntingtin mutations should be routine.

P12.109 A novel PCBD gene mutation in an Iranian patient with HyperphenylalaninemiaM. Raeisi¹, N. Mahdieh², H. Bagherian¹, R. Vahidi¹, M. Masoudifard¹, S. Zeinali^{1,3};¹Kawsar research center, Tehran, Islamic Republic of Iran, ²Ilam University of Medical Sciences, Ilam, Islamic Republic of Iran, ³Dep't of Mol. Med., Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Islamic Republic of Iran.

Neonatal screening for PKU is carried out nationally and our center is one of the referral centers for molecular analysis of PKU in Iran. Hyperphenylalaninemias are common disorders of phenylalanine catabolism. Six genes including PAH, PTPS, DHPR, GTPCH, SR and PCBD independently play role in this disorder.

A 2-year-old boy was referred to our center for genetic diagnosis of PKU. PAH gene was sequenced but no mutation was found. Using STR based linkage mapping approach, BH4-metabolizing genes were screened. Pattern of autozygosity by descent (ABD) suggested that PCBD gene may be involved in this family. This gene was sequenced and a homozygous T>C substitution (X105Q) was found in the termination codon.

P12.110 Fine-scale Survey of X Chromosome Copy Number Variants Underlying Intellectual DisabilityA. Whibley¹, V. Plagnol^{1,2}, P. Tarpey³, F. Abidi⁴, T. Fullston^{5,6}, M. Choma¹, C. Boucher¹, L. Shepherd¹, L. Willatt⁷, G. Parkin⁸, R. Smith³, P. Futreal⁸, M. Shaw⁵, J. Boyle⁸, R. Stevenson⁴, G. Turner⁸, A. Hackett⁸, M. Field⁸, C. Schwartz⁴, J. Gecz^{5,6}, M. Stratton³, F. Raymond¹;¹Cambridge Institute for Medical Research, Cambridge, United Kingdom,²University College, London, United Kingdom, ³Wellcome Trust Sanger Institute, Cambridge, United Kingdom, ⁴J.C. Self Research Institute of Human Genetics, Greenwood, SC, United States, ⁵Women's and Children's Hospital, Adelaide, Australia, ⁶University of Adelaide, Adelaide, Australia, ⁷Addenbrookes Hospital, Cambridge, United Kingdom, ⁸Hunter Genetics Service, Waratah, Australia.

Copy number variants in 251 families with evidence of X-linked intellectual disability (XLID) were investigated by array comparative genomic hybridization on a high-density oligonucleotide X chromosome array platform. We identified pathogenic copy number variants in 10% of families, with mutations ranging from 2kb-11Mb in size. The challenge of assessing causality was facilitated by prior knowledge of XLID-associated genes and the ability to test for co-segregation of variants with disease through extended pedigrees. Fine-scale analysis of rare variants in XLID families leads us to propose four additional genes, PTCHD1, WDR13, FAAH2 and GSPT2, as candidates for XLID causation and the identification of further deletions and duplications affecting X chromosome genes but without apparent disease consequences.

Breakpoints of pathogenic variants were characterised to provide insight into the underlying mutational mechanisms and indicated a predominance of mitotic rather than meiotic events. By effectively bridging the gap between karyotype-level investigations and X chromosome exon re-sequencing, this study informs discussion of alternative mutational mechanisms, such as non-coding variants and non-X linked disease, which might explain the shortfall of mutation yield in the well-characterised IGOLD cohort, where currently disease remains unexplained in two thirds of families.

P12.111 Jervell and Lange Nielsen syndrome a new manifestation of p.S38G in KCNE1 gene.M. Farhadi¹, M. Falah¹, M. Houshmand², O. Aryani³, H. Emamjomeh¹, A. Asghari¹;¹Department and Research Centre of ENT & Head and neck Surgery of Iran Medical University, Tehran, Islamic Republic of Iran, ²National Institute for Genetic Engineering and Biotechnology, Tehran, Islamic Republic of Iran,³Medical Genetic Laboratory Special Medical Centre., Tehran, Islamic Republic of Iran.

Autosomal recessive Jervell and Lange Nielsen syndrome (J-LN) is a condition that causes profound hearing loss and disruption of heart's normal rhythm. J-LN caused by homozygous or compound heterozygous mutations on the KCNQ1 or on the KCNE1 genes encoding the I_{Ks} current channel.

An 8-year-old girl was referred to the Department and Research centre of ENT because of sensorineural hearing loss and twice history of fainting episodes and QT prolongation 527 msec and with consanguineous parents.

Genomic DNA was obtained from peripheral blood, screened KCNE1 mutation with direct sequencing.

Direct sequencing showed P.S38G that caused serine by a glycine residue at amino acid position 38, this change reported before separately with noise-induced hearing loss and in patient with Atrial Fibrillation that here for the first time represented with Jervell and Lange Nielsen syndrome.

Due to Jervell and Lange Nielsen syndrome is a reason for young syncope so examination of presence of KCNE1 gene mutation can usefully contribute to diagnosis and medical from preventing death.

P12.112 Tackling rare diseases using new possibilities; KFSD and TOD crackedJ. T. den Dunnen¹, R. Al-Moman², Y. Sun², E. Aten², E. Bakker², M. H. Breunig²;¹Leiden Genome Technology Center, Human & Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands, ²Human & Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands.

Due to recent developments in sequencing technology (next generation sequencing - NGS) the cost to sequence large genomic regions, full human exomes and even complete genomes is rapidly decreasing. We have applied these new possibilities to tackle unresolved cases of monogenetic diseases where cases are rare, families too small and/or candidate gene regions were too large. Regions of interest were targeted by PCR amplification and/or hybridisation capture (on array / in solution) using custom-design or X-chromosome exome probe sets. The first case cracked was Keratosis Follicularis Spinularis Decalvans (OMIM 308800), a rare genetic disorder affecting both skin and eyes. Sequencing of genes in the candidate region implicated only mutations in the MBTPS2 gene as causing KFSD. Other mutations in this gene have been recently shown to cause IFAP syndrome (OMIM 308205), a disease with partially overlapping phenotype. A second case was Terminal Osseous Dysplasia (OMIM 300244), a rare male-lethal X-linked dominant disease characterized by skeletal dysplasia of the limbs, pigmentary defects of the skin, and recurrent digital fibroma during infancy. Pathogenic mutations were found in only 1 gene in the candidate region with all unrelated patients studied so far having an identical mutation. The variant X-chromosome was fully inactivated in all samples analysed, making it difficult to prove the pathogenic effect of the mutation, suggested to affect splicing. Further diseases are under analysis; our progress will be reported at the meeting.

P12.113 Molecular genetic evaluation of patients with Klinefelter syndrome

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The Klinefelter's syndrome (KS) is the most common chromosomal disorder characterized by testicular dysgenesis, male hypogonadism and infertility. The aim of our study was to evaluate genetic factors that may be involved in phenotypic variability in KS patients.

Materials and Methods: We examined 20 non-mosaic KS patients. The Y-microdeletions and the androgen receptor (AR) gene were analyzed. Classic AZF deletions were tested according to EAA/EMQN guidelines (1999). Partial AZFc deletions were detected by mPCR of following STSs: sY142, sY1197, sY1192, sY1291, sY1206, sY1054 and sY1125. PCR with primers flanking (CAG)n polymorphic region in exon 1 of the AR gene and methylation-sensitive restriction with enzyme HpaII were used to determine CAG repeat length and the X-inactivation.

Results: No complete AZF deletion was found. Partial AZFc deletions were detected in 15% patients. All individuals presented Yq microdeletions were azoospermic. In two patients the b2/b3 deletion was found, in the other one rare partial AZFc deletion was detected. The mean CAG repeat number was 21.25. Only 42% patients were found to be the heterozygotes. Non-random X- inactivation was revealed in these individuals. Long AR (CAG)n repeat allele (n>26), was detected only in 2.6% examined chromosomes.

Conclusions: Our study demonstrated an absence of classic AZF deletions in KS patients. Partial AZFc deletions were found out with enough high frequency. Non-random X-inactivation and long AR (CAG)n alleles apparently are rare in KS patients. The further large cohort studies will allow more accurately assessing a frequency of partial AZFc deletions and their significance in KS patients.

P12.114 Familial Hypercholesterolemia in the Czech Republic

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Familial hypercholesterolemia (FH) is an autosomal dominant disorder caused by mutations and large rearrangements in the gene encoding the low-density lipoprotein receptor (*LDLR*). The frequency of heterozygotes is 1/500. The frequency of homozygotes or compound heterozygotes is 1/1 000 000.

In the set of Czech patients with FH, we found 75 types of causal small DNA rearrangements (18 of them were not described so far) and 9 types of large DNA rearrangements (6 of them were not described so far) in *LDLR*. Large DNA rearrangements were analyzed using MLPA. Using long-range PCR, PCR, and DNA sequencing, we analyzed breakpoints of deletions/duplications identified in our FH patients. In 8 rearrangements, we characterized their exact extent and breakpoint sequences. The results showed that 6 events were products of unequal allelic homologous recombination (NAHR) between *Alu* repeat sequences. The remaining 2 events apparently originated from non-homologous end joining (NHEJ). NHEJ has not been described in relation to *LDLR* up to now.

From 1253 FH probands, causal events were found in 476 of them. Further, we designed genotyping microarray based on the technology APEX (Arrayed Primer Extension) that enable detection of 75 mutations found in the Czech population and 75 most common mutations from another European population. The validation results indicate that the FH chip seems to be a suitable tool for the first line screening of mutations in the *LDLR* gene.

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P12.115 Identification of novel mutations in LCA5 gene by genome-wide homozygosity mapping in patients with Leber congenital amaurosis

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Purpose: Leber Congenital Amaurosis (LCA) is the earliest and most severe form of all inherited retinal dystrophies, responsible for congenital blindness. To date, 14 LCA-associated genes have been identified by linkage analysis, homozygosity mapping and/or candidate gene analysis: AIPL1 (17p13.1), CEP290 (12q21.3), CRB1 (1q31-q32.2), CRX (19q13.3), GUCY2D (17p13.3), IMPDH1 (7q31.3-q32), LCA5 (6q14.1), LRAT (4q32.1), RD3 (1q32.3), RDH12 (14q23.3-q24.1), RPE65 (1p31), RPGRIP1 (14q11), SPATA7 (14q31.3) and TULP1 (6q21.3). Mutations in these genes account up to 70% of the LCA patients, suggesting that additional causative mutations in new and/or previously known genes remain to be identified. In this study, we have used a genome-wide homozygosity mapping strategy to identify novel mutations in LCA individual with inbred or outbred background.

Material And MethodS: Patients from a Spanish cohort of 28 recessive LCA families were genotyped with GeneChip 500K Affymetrix SNP microarrays. Known mutations in eight LCA genes were previously excluded with a genotyping microarray based on arrayed primer extension (APEX) technology.

Results: Several homozygous segments were identified in all the 28 patients, regardless of the absence of proven consanguinity. Eight families (28%) shared a homozygous region at chromosome 6q13-14.1, encompassing the *LCA5* locus. By direct sequencing, two novel homozygous mutations were identified in *LCA5* gene in two of the eight families: 1) a frameshift p.Glu132fsX5 and 2) a missense p.Ser202Pro. Both variants were not found in a Spanish cohort of 100 healthy individual controls.

Conclusions: This study underlines homozygosity mapping as a useful approach to identify novel pathogenic mutations in LCA molecular diagnosis.

P12.116 Homozygosity mapping in a Turkish family with Leber Congenital Amaurosis by high density SNP microarray

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Leber Congenital Amaurosis (LCA) is one of the most severe hereditary eye disease that represent genetic cause of congenital visual impairment in infants and children. LCA is a genetically heterogeneous condition, consisting of a group of autosomal recessive retinal dystrophies. Recent molecular genetic studies have linked 12 genes (AIPL1, CEP290, CRB1, CRX, GUCY2D, LCA5, RD3, RDH12, RPE65, RPGRIP1, TULP1, LRAT) to LCA. Difficulties in clinical classification of LCA cases can cause misdiagnosis. Beside this, the genetic heterogeneity and complexity of several LCA genes has hampered routine molecular analysis. High-throughput molecular screening techniques are necessary to surmount this issue.

In this study, whole genome genotyping was performed by Affymetrix 250K SNP arrays in a consanguineous Turkish family in which four children have macular coloboma type LCA. Homozygosity mapping have shown linkage to chromosome 14 in this family. LCA causing RDH12 gene located in this chromosomal region was selected as a first candidate gene for mutation screening. In four patients, Thr49Met mutation in exon 2 was identified by direct sequence analysis in RDH12 gene. RDH12 mutations account less than 3% of all LCA-associated gene mutations. Therefore, RDH12 gene is not primarily preferred among other responsible genes for mutation screening studies in LCA. In large LCA families, homozygosity mapping could be used as a rapid identification of disease causing locus and molecular diagnosis.

P12.117 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 progressive involvement of proximal limb girdle muscles.

Limb girdle muscular dystrophy type 2A (LGMD2A) is an autosomal recessive disorder characterized by atrophy and weakness of proximal girdle muscles. LGMD2A is caused by mutations in the *CAPN3* gene (15q15) that encodes the muscle specific protein, calpain-3 (p94). LGMD2A is the most frequent form of LGMD in many European countries.

Another relatively common form of LGMD is LGMD2I 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.

We performed analysis of the *CAPN3* gene and *FKRP* gene 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 175 unrelated patients for mutations in the *CAPN3* gene. 40 patients (23%) were found to carry mutations in the *CAPN3* gene. We detected 16 previously reported mutations and 3 novel mutations (c. 802_945del, c. 1783_1788del, p.Q619X). Our results show that mutation 550delA is the most frequent *CAPN3* defect in Czech LGMD2A patients (53%). We screened 76 unrelated patients for the most common mutation in the *FKRP* gene (p.Leu276Ile). The mutation was found in 5 patients (7%).

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P12.118 The frequency of Limb Girdle Muscular Dystrophy 1C in southern Italy

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Caveolin-3 (Cav3) is a protein composed of 151 amino acids mainly expressed in skeletal muscle tissue where it seems to play a key role in the maintenance of plasma membrane integrity. In addition caveolin-3 has been implicated in signal transduction and vesicular trafficking. Mutations in the gene encoding caveolin-3 (CAV3), located on chromosome 3p25, were first identified in patients with autosomal dominant limb girdle muscular dystrophy (LGMD1C), hyperCKemia, rippling muscle disease and distal myopathy. Mutations in CAV3 gene were also reported in long QT syndrome and in Sudden infant death syndrome. Recently, missense mutations in CAV3 gene were associated with an autosomal recessive LGMD phenotype. To evaluate the frequency of LGMD1C in southern Italy we performed the molecular analysis of CAV3 gene in clinically suspected LGMD patients. The exons and flanking intronic boundaries of the gene were analysed by sequencing. Among the screened 50 LGMD patients we identified only a patient with a heterozygous 3bp microdeletion (290-293del) in exon 2 of CAV3 gene, resulting in loss of a phenylalanine (Phe97del). This mutation has been previously identified by other authors in an Italian family. The autosomal dominant forms of LGMD are relatively rare and represent probably less than 10% of all cases. Our findings indicate that the frequency of the LGMD1C in southern Italy is around 2%. Our future plan is to perform the molecular analysis of the other genes involved in the pathogenesis of LGMD in order to evaluate the relative proportion of the different types of LGMD in southern Italy.

P12.119 Novel mutations in *CAPN3* and *LAMA2* genes

associated with genetic and phenotypic heterogeneities within a single consanguineous family involving both congenital and progressive muscular dystrophies.

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Limb girdle muscular dystrophy (LGMD) and congenital muscular dystrophy (CMD) are two common forms of neuromuscular disorders which are distinguishable by their age of onset but with probably similar underlying pathway. In this study, we report the immunohistochemical, western blot and genetic analyses in a large consanguineous Tunisian family with two branches including seven patients sharing similar LGMD2 phenotype in one branch and one CMD patient in the second. Linkage analyses were compatible with the LGMD2A locus in one family branch and with MDC1A locus in the other one. This result

was supported by deficiency in merosin and calpain3 in the CMD patient and LGMD patients respectively. Mutation analysis revealed two distinct mutations: a c.8005delT frameshift deletion in exon 56 of the *LAMA2* gene (MDC1A) was found in the CMD patient and a new homozygous mutation c.1536+1G>T in the donor splice site of intron 12 of the *CAPN3* gene (LGMD2A) was found in the LGMD patients for the first time. RTPCR performed on total RNA from a LGMD2A patient's muscle biopsy showed a complete retention of intron 12 in *CAPN3* cDNA generating a premature termination codon which potentially elicits the nonsense mRNA to degradation by NMD (nonsense-mediated mRNA decay). Our data indicate that mRNA analysis is necessary to clarify the primary effect of genomic mutations on splicing efficiency that alters mRNA processing and expression level.

P12.120 Establishing web-based gene variant databases for all Mendelian disorders

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The EU-funded Gen2Phen project (<http://www.Gen2Phen.org/>) aims to provide advanced informatics solutions to link genotypes to phenotypes. One deliverable is to establish web-based gene sequence variant databases (LSDBs) for all human genes involved in Mendelian disorders. This should provide all those involved an easy, cheap and effective way to share their findings and use the full collection to draw conclusions on the potential phenotypic consequences of a given variant, e.g. „pathogenic or not“ when in a clinical setting. Recently, a study was completed that sequenced the coding exons of all genes on the X chromosome in 208 families with X-linked mental retardation. All sequence variants identified were stored on a gene-by-gene basis in a single LSDB installation containing 542 genes (<http://www.LOVD.nl/IMR>). The platform used, LOVD, allows easy web-access, submitting and curating variants and viewing and querying of the collected information. Individual databases can be customized to specific user needs, directly link to other sources (internet, intranet, local-PC files) and exchange data with central repositories. By mid-2010, the LSDBs established will be extended to cover all human genes involved in Mendelian disorders. To make this resource most useful, we invite clinicians and researchers working on genetic disorders world-wide to submit their findings. In addition we solicit volunteers to become a gene guardian (curator), checking incoming information and thereby ensuring data quality.

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P12.121 Contributions of alternative splicing to transcript diversity: novel variants of Machado-Joseph Disease gene (ATXN3)

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Machado-Joseph disease (MJD) is a late onset neurodegenerative disorder that presents clinical heterogeneity, not completely explained by its causative mutation. MJD is caused by an expansion of a CAG tract at exon 10 of the *ATXN3* gene (14q32.1), which encodes for ataxin-3. The main goal of this study was to analyze the occurrence of alternative splicing at the *ATXN3* gene, by sequencing a total of 415 cDNAs clones (from 20 MJD patients and 14 controls). Two novel exons were described for the *ATXN3* gene. Fifty-six alternative splicing variants, generated by 4 types of splicing events, were observed. From those variants, 50 were not previously described and 26 were only found in MJD patients samples. Most of the variants (85.7%) present frameshift, which leads to the appearance of premature stop codons. Thirty-seven of the observed variants constitute good targets to nonsense mediated decay. The remaining variants are likely to be translated into

at least 20 different isoforms. According to the presence/absence of known ataxin-3 domains, it can be predicted that some of those variants may contribute to increased toxicity, while others may be protective. Findings from this work confirm that alternative splicing constitutes an important mechanism regulating protein diversity and indicate that there is genetic variability, other than that found in genomic DNA, which may be enrolled in the MJD pathogenic process, as well as in the neuropathology of other polyglutamine disorders.

P12.122 Clinical and genetic studies of Tunisian and Algerian families with Mal de Meleda disease

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Mal de Meleda MDM (OMIM 248300) is a rare autosomal recessive palmoplantar keratoderma (PPK) with prevalence of 1 in 100000. Clinically, it is characterized by transgressive PPK, particularly over the joints, perioral erythema, brachydactyly and nail abnormalities. The progressive lesion can lead to a severe functional handicap with reduced mobility of hands and feet including spontaneous amputation of digits. It is caused by mutations in *SLURP1* gene located in chromosome 8 (8q24.3).

Here we investigate 2 consanguineous families. The first is Tunisian with one affected women and the second is Algerian with 2 affected children. A detailed disease history with the age of onset, distribution, and clinical course of their skin lesions were noted and a full clinical examination was performed for every patient. We proceed to direct sequencing of the 3 exons in the *SLURP1* gene. Sequence analysis revealed 2 known mutations in exon 2; **c.82delT** in the Tunisian family and **+1 IVS2 G>A** for the Algerian family.

P12.123 Medullary cystic kidney disease: further evidence of genetic heterogeneity based on a large Portuguese family

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Background: Medullary cystic kidney disease type 2 (MCKD2) is a rare autosomal dominant syndrome characterized by gout, tubulointerstitial nephropathy and end-stage renal failure (ESRF). The disease is caused by mutations in the uromodulin gene (UMOD, 16p12.3). Besides, MCKD1, with overlapping phenotype to MCKD2, but milder symptoms and with later age-at-onset is associated with a candidate disease locus on chromosome 1q21.

Patients and Methods: We investigated a Portuguese family of 4 generations suffering of MCKD2 syndrome. The proband of the family developed ESRF at the age of 28 years. Further nine family members were affected. The disease showed a typical autosomal dominant trait in the family. We tested the entire coding region of the UMOD gene for mutations with PCR and direct sequencing analysis.

Results: Affected family members showed elevated serum uric acid levels. Renal biopsy, carried out in one affected family member revealed signs of a tubulointerstitial disease. Immunohistochemistry of the kidney showed staining of uromodulin in tubule profiles. Genetic analysis of the UMOD gene showed three non-pathogenic variants but did not reveal any pathogenic mutations.

Conclusion: The clinical and pathological characteristics of this family suggest a clear MCKD2 syndrome. The missing of pathogenic mutations in the UMOD gene points out the genetic heterogeneity of this disease and broadens the phenotypic spectrum of non-UMOD related MCKD. Further investigations of this family might be helpful in identifying candidate genes for MCKD1 and describe genotype-phenotype correlations.

P12.124 Monocarboxylate transporter 10 (MCT10) implications in oligodendrocyte T3 transport and in dysmyelinating phenotypes

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Thyroid hormones (TH) are known to be essential for proper brain development, notably for myelination by controlling oligodendrocyte (the central nervous system (CNS) myelinating cells) differentiation. Nevertheless, nothing is known about TH transporters in oligodendrocytes. We reported that patients with mutations in the TH transporter monocarboxylate 8 (MCT8) display not only an X-linked mental retardation associated with spastic paraparesis but also a myelination delay. This observation suggested that MCT8 participates in TH transport in oligodendrocytes and we demonstrated that this is the case in human and mouse oligodendrocyte cell lines.

Another member of the MCT family, MCT10, has recently been identified as a new TH transporter expressed in the mouse CNS in white matter structures where oligodendrocytes are localized. We therefore sought whether MCT10 could play a role in TH transport in oligodendrocytes and whether MCT10 mutations could be responsible for dysmyelinating phenotypes. We established that MCT10 is implicated in T3 transport, at least in the human oligodendrocyte cell line MO3.13, since MCT10 silencing using siRNA impairs 25+/-8.02% of T3 transport. Together, MCT8 and MCT10 account for at least 63+/-2.6% of T3 transport in MO3.13. We are now looking for MCT10 mutation in dysmyelinating phenotypes of unknown origin in a cohort of 45 patients presenting a myelination defect at birth. Results from the sequencing of the 6 coding exons of the MCT10 gene will be presented.

These results will contribute to better define how TH are transported into oligodendrocytes and to assess whether MCT10 could be implicated in human pathology.

P12.125 Identification of an X-chromosomal microdeletion in a patient suspected of the Allan-Herndon-Dudley Syndrome using a dense SNP array

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Thyroid hormone (TH) is crucial for the development of different organs, in particular the brain. Different TH transporters have been identified including monocarboxylate transporter 8 (MCT8). The clinical importance of TH transporters is shown in patients with mutations in *MCT8* (*SLC16A2*). The patients suffer from severe X-linked psychomotor retardation in combination with disturbed TH levels, especially high serum T3 levels, now referred to as Allan-Herndon-Dudley Syndrome (AHDS).

A male patient with severe psychomotor retardation, elevated T3 serum levels and suspected of having AHDS was referred to our lab for genetic studies. PCR analysis of *MCT8* showed the presence of exon 1, while exons 2-6 were missing. We used the Illumina HumanHap 610-Quad array to determine in the patient the approximate size of the deletion downstream of *MCT8* and if the deletion comprised other genes. DNA of the mother and two randomly selected female controls were also investigated. There was a complete loss of 9 SNPs involving 45.5 kb around *MCT8* which was confined to the DNA of the patient. Further analysis by PCR-based mapping revealed an X-chromosomal deletion between 59.6 and 82.7 kb.

In conclusion, using a high-density SNP array we could determine that the deletion in the patient was limited to *MCT8* and of likely *de novo* origin considering that the mother was not a carrier of this X-chromosomal deletion. We confirmed that the patient is an AHDS patient with a large *MCT8* deletion, which probably explains his phenotype by the suggested lack of *MCT8* expression.

P12.126 MEV mutations in Iranian patients suffering from familial Mediterranean fever: analysis of 12 mutations

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Familial Mediterranean fever (FMF) is an autosomal-recessive inherited inflammatory disorder. It is characterized by recurrent episodes of painful inflammation in the abdomen, chest or joints. The responsible

disease gene, designated *MEFV*, has been mapped on chromosome 16p13, comprises of 10 exons and encodes a protein called marenostrin or pyrin, which is found in white blood cells. Because of non-specific clinical symptoms, the molecular genetic analysis of *MEFV* significantly improves early and precise FMF diagnosis.

In this study we used multiplex PCR and reverse-hybridization (FMF StripAssay, ViennaLab Diagnostics) to analyze the following 12 *MEFV* mutations: E148Q, P369S, F479L, M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S and R761H in 250 Iranian patients, which were referred to us based on clinical criteria indicating FMF.

We identified *MEFV* mutations in 60.4% of our patients. Out of these mutations, 83.5% were located in exon 10, while the remaining 16.5% were found in other exons. The most common mutation was M694V (42.3%), which is known to be a founder mutation in other populations. In 39.6% of patients we could not identify a mutation that could explain their clinical status. Some of our patients were sent for comprehensive *MEFV* sequencing, but no additional mutation was identified.

P12.127 X-linked hypophosphatemic rickets with audio-vestibular symptoms - a model for understanding Meniere's disease?

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The etiology of Meniere's disease (MD) is unknown. Some individuals with X-linked hypophosphatemic rickets (XLH) exhibit audio-vestibular symptoms similar to MD. We hypothesized that XLH patients may be investigated in order to gain insight in the biochemical disturbances underlying MD. A three-generation XLH-family with a known *PHEX*-gene mutation was studied. Some individuals with XLH had audio-vestibular symptoms, some did not. Past phosphate substitution, blood laboratory tests, audiological tests and inner ear images (CT and MRI) were studied in several affected family members. The differences found in the study of these XLH individuals, with and without audio-vestibular symptoms, will be presented.

P12.128 Molecular genetic testing of mental retardation in Bulgaria

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Mental retardation is highly heterogeneous in clinical and genetic point of view. Here we discuss on: Fragile X syndrome (FXS), Rett syndrome (RTT), Prader-Willi/Angelman syndromes (PWS/AS) and microdeletion syndromes (MDS). The genes studied are: FMR1, MECP2, CDKL5, ARX, and methylation analysis of exon 1 of the SNRPN. Multiplex ligation-dependent probe amplification (MLPA) analysis was used to look for large deletions/duplications, to screen for microdeletion syndromes, and to clarify methylation status of FMR1 and SNRPN genes. Our results in the group of FXS revealed about 12% mutations in the FMR1 gene. A mosaic case full mutation/normal allele was detected. The genetic tests in the group of RTT girls revealed about 26% mutations along the MECP2 gene. No CDKL5 gene mutations were detected. The genetic tests in the group of PWS/AS revealed 64% mutations along the 15q11-q13 region. The MLPA analysis in the group of MDS revealed genetically confirmed cases with diagnosis Williams Beuren and Wolf-Hirschhorn syndromes. Altogether, we manage to clarify the molecular basis in about 30% of our mentally retarded patients. The percentage of genetically proved diagnosis among our RTT patients, PWS/AS and MDS cases is relatively high, which represents a good clinical recognition of these pathological entities. On the contrary, the percentage of the genetically confirmed FXS cases is relatively low. Most probably this is due to the characteristics of this group, being clinically mixed and containing some definite autistic cases.

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P12.129 The Arg468His mutation in MFN2 is the most frequent cause of CMT2A in Spanish Patients

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Introduction: The most common form of axonal Charcot-Marie-Tooth disease (CMT) is type 2A, caused by mutations in mitochondrial GTPase mitofusin 2 (MFN2).

Objective: To establish the incidence of MFN2 mutations in a cohort of Spanish patients with axonal CMT neuropathy.

Material and Methods: We studied 85 families with suspected axonal CMT. All MFN2 exons were studied through direct sequencing. A bioenergetics study in fibroblasts was conducted using a skin biopsy taken from a patient with an Arg468His mutation.

Results: Twenty-four patients from 14 different families were identified with nine different MFN2 mutations. MFN2 mutations were responsible for CMT2 in 16% \pm 7.7% of the families studied and in 30.8% \pm 14.2% (12/39) of families with known dominant inheritance. The Arg468His mutation was the most prevalent (6/14 families). A bioenergetics study in fibroblasts was conducted using a skin biopsy taken from a patient with this mutation. These bioenergetic studies showed the typical results of MFN2 patients.

Conclusion: The Arg468His mutation is the most prevalent (6/14 families) mutation in MFN2 causing CMT2A in Spanish patients and our study confirmed that it is actually pathological, presenting as a neuropathy in a mild to moderate degree.

P12.130** Role of the microRNA-183 family in the pathogenesis of hereditary nonsyndromic hearing loss in the Italian population

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The mir-183 microRNA (miRNA) family is composed of mir-183, mir-96, and mir-182, which are coordinately expressed from a single genetic locus in vertebrates. This highly conserved miRNA family is essential for differentiation and function of the vertebrate inner ear. In 2009, 2 mutations in the human MIRN96 gene have been reported in 2 Spanish families affected by autosomal dominant nonsyndromic sensorineural hearing loss (AD-NSHL). This represented the first evidence implicating a point mutation within a miRNA in a Mendelian disease. We screened a total of 770 NSHL patients and 808 normal-hearing Italian controls for mutations in MIRN96, MIRN182, and MIRN183. Neither of the 2 previously reported MIRN96 mutations were found, suggesting that they might represent private mutations. Instead, at least one putative novel mutation within mir-96, +57T>C, was identified in a patient with a family history of AD-NSHL. The detected variant replaces a highly evolutionarily conserved nucleotide located outside the mature mir-96, and is predicted to reduce the stability of the pre-miRNA secondary structure (dG from -34.4 to -30.4 Kcal/Mol). Moreover, the variation occurs within the seed region of the mature mir-96*, which is processed from the complementary strand of the mir-96 precursor. The mir-96* seem to have been maintained throughout vertebrate evolution, although its sequence is only partially conserved. Very little is known about mir-96* expression and function. The effect of mir-96+57T>C on mir-96 processing as well as on mir-96* processing/target recognition is currently underway by ex-vivo expression experiments in human cell lines.

P12.131 Severe mitochondrial DNA depletion in infancy.

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Mitochondrial DNA (mtDNA) copy number reduction, known as the mitochondrial DNA depletion syndrome (MDS), is a common cause of severe mitochondrial disorders of infancy and early childhood. MDS results from defects in nuclear encoded factors involved in mtDNA maintenance and within the past years mutations in the *POLG1*, *DGUOK*, *MPV17*, *PEO1*, *SUCLG1*, *TK2*, *SUCLA2* and *TYMP* genes have proven to be implicated in the pathogenesis of this disorder. The clinical phenotypes associated with the different gene alterations vary considerably but present either as a hepatocerebral or a myopathic syndrome.

We have identified a homozygous p.E85 deletion in exon 3 of the *RRM2B* gene in a neonate. The patient, born to first-cousin Caucasian parents, presented with lactic acidosis, severe hypotonia, deafness, blindness and hyperammonemia. Muscle biopsy showed RRF, a combined respiratory chain defects and massive subcomplexes of ATP synthase both with traditional spectrophotometry and BN-PAGE. Western blotting using antibodies against selective OXPHOS subunits indicated the preservation of nuclear encoded complex II. She died at 2 months of age. Mutations of the *RRM2B* gene, encoding the cytosolic R2 subunit of a p53 controlled ribonucleotide reductase (p53R2), have been reported to cause severe depletion of muscle mtDNA by Bourdon et al. 2007. Indeed, < 5% residual amount of mtDNA was measured in muscle tissue of our patient. Aberrations in these 9 genes count only for a minority of all MDS cases. It is expected that other genes involved will be identified soon.

P12.132 Motor chip: a CGH microarray for neuromuscular disorders

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Neuromuscular disorders are a highly heterogeneous group of genetically determined diseases in which at least 245 different genes have been involved (www.musclegenetable.org).

We developed a specific array CGH assay, called the "Motor chip". The Motor chip includes 465 genes selected for (i) their involvement in Mendelian neuromuscular disorders (245), (ii) their involvement in muscle specific metabolic pathways and/or functions (97), (iii) their muscle-specific expression level (48), or (iv) the putative interaction of their protein product with LGMD genes (75).

The selected genes correspond to 42,665,641 bp of genomic sequence and to 3,543,193 bp of coding sequence that was covered by selecting 38,481 specific 60 bp long probes (coverage 99.8%). The design was developed using the Agilent platform and the 8X60K format, representing the most cost-effective option. The free surface of the array was randomly filled with probes from Agilent Human Genome 44K CGH array, covering the whole human genome.

Validation experiments were carried out using DNA samples from dystrophic patients in which deletions/duplications had previously been identified in different genes (DMD, CAPN3, and SGCG). All the aberrations were correctly addressed.

We next tested 24 samples from LGMD patients for which no mutation was found. The Motor chip identified one patient with two complex SGCG gene deletions, one patient with a DYSF gene duplication involving the first 5 exons and other minor aberrations. A second generation of the Motor chip is currently under development. We consider that this tool may be very useful for the diagnosis of all unknown neuromuscular disorders.

P12.133 Analysis of *COL1A1* gene in Osteogenesis Imperfecta patients in Bashkortostan Republic of Russia

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Osteogenesis imperfecta (OI) is a clinically and genetically heterogeneous disorder of connective tissue. We carried out the analysis of *COL1A1* gene in 36 patients from 30 families with OI from Bashkortostan (Russia). Two nonsense mutations (Arg361X and Arg415X) in *COL1A1* gene have been detected using SSCP analysis. Both mutations convert CGA which encodes arginine to TGA that results in a premature stop codon (causes a 50% reduction of collagen produc-

tion). In literature this mutation was described as the cause of mild OI type I with an autosomal dominant inheritance pattern. In our sample this Arg361X mutation resulted in severe OI type III. The c.1243 C>T mutation (Arg415X) also causes premature termination codon. Three polymorphisms in the promoter region (-1997G/T, +1245G/T and -1663indelT) of *COL1A1* gene were shown to be associated with the bone mineral density. Analysis of -1997G/T polymorphism revealed statistically significant differences in genotype frequencies distribution between the patients with OI (29) and the control group (150): -1997G/T genotype was shown to be the factor risk of fracture development (OR=2.38, 95%CI 1.06-5.34). While -1997G/G genotype might be considered as protective one (OR=0.4, 95%CI 0.19-0.98). Statistically significant differences were demonstrated with respect to genotype combinations of *COL1A1* polymorphisms -1997G/T, -1663indelT and +1245G/T between individuals with OI and control group. Genotypes combination -1997G/T+ -1663indelT+1245G/T was associated with high risk of fracture development (OR=11.84, 95%CI 2.06-68.09), whereas combination -1997G/G+ -1663indelT+1245G/G - was shown to be protective (OR=0.45, 95%CI 0.22-0.95).

P12.134 V851M mutation in the *CLCN1* gene causes recessive Myotonia Congenita

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Myotonia Congenita (MC), one of the most common forms of non-dystrophic types of myotonia, may show both dominant (Thomsen disease) and recessive (Becker disease) modes of transmission, both of which may be caused by mutations in *CLCN1*(7q35), which encodes a chloride channel that is present in skeletal muscle. In this study we describe 2 Arab families with MC, from two different villages in northern Israel. The first family was referred to genetic counseling because of an undefined myotonia in a child. The family tree showed a recessive pattern of heredity. The entire *CLCN1* gene was sequenced and a change in the coding region of exon 22 was found. The 2551G>A substitution causes a missense mutation V851M, located in the conserved CBS2 domain in the C terminus of the *CLCN1* channel. All of the affected children in the extended family were found to be homozygous to the V851M mutation, while their parents and the healthy siblings were found to be heterozygous to the mutation. The second family was previously described by us (Muscle Nerve. 2009 Aug 20). In this family a novel missense mutation [568GG>TC (G190S)] was found in twelve members which transmitted the condition in an autosomal dominant manner with incomplete penetrance.

P12.135 Myotonia congenita: identification of new mutations in the *CLCN1* gene

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Myotonia congenita (MC) is a hereditary muscle disorder characterized by delayed relaxation of skeletal muscle after voluntary contraction (myotonia) that can be inherited either as an autosomal-dominant (Thomsen Disease, OMIM 160800) or recessive trait (Becker Disease, OMIM 255700). The clinical features of the two conditions are very similar but can be distinguished by severity and inheritance pattern. Both disorders are caused by mutations in the *CLCN1* gene on chromosome 7q35, encoding the skeletal muscle chloride channel CIC-1. In the general population, the incidence is reported as 1:23.000 live births for the dominant form and as 1:50.000 for the recessive form. To date 137 mutations have been described in literature. In the present study, we analysed for mutations the *CLCN1* gene in a panel of 101 clinically well-characterized unrelated patients originating from different countries (69 males and 32 females). Detailed clinical charts were collected for each patient. Mutation analysis was conducted by DHPLC screening of all coding exons followed by direct sequencing. We identified 54 different mutations in 67 patients (66%), of which 27 were novel. The mutation spectrum in our analysis was composed of 31 missense, 12 non-sense, 6 splicing and 5 frameshift mutations.

Among the 101 investigated patients 45 (44%) were homozygous or compound heterozygous for two *CLCN1* mutations, 22 (21%) were heterozygous for one mutation and 34 (33%) were negative. The present study expands the *CLCN1* gene mutation spectrum and will contribute to further understand the molecular basis underlying the MC phenotypic variability.

P12.136 Myotonic dystrophy type 2 in Russian families

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Myotonic dystrophy (dystrophia myotonica, DM) is genetically heterogeneous. Along with 'classic' widespread DM1 caused by expansion of CTG-repeats in *DMPK* gene there exists an infrequent DM2 caused by expansion of CCTG-repeats in zink finger protein 9 gene (*ZNF9*) and found exclusively in European populations. DM1 and DM2 share main features though DM2 has some distinctions: mean age of onset is 48 yrs, muscle weakness is predominantly proximal in contrast to distal myopathy in DM1, myalgia is a characteristic feature, cardiomyopathy is rare and cognitive disorders are not seen while other extramuscular features are similar to DM1; anticipation is not typical, and congenital forms do not exist, but reverse anticipation is possible. We present first Russian DM2 cases. In one family the mother and the daughter were affected at the age of 21 and 15 accordingly. Daughter at the age of 21 had weakness only in muscles of brush. Mother at the age of 60 had hypotrophy of muscles of brush and footstep. In the second family a 55-year-old woman was an only patient. Weakness became apparent in 50 yrs, myopathy was predominantly distal in upper and diffuse in lower limbs, myotonia was mild, intractable myalgia was a prominent symptom, extramuscular features were cataracts and hearing loss. We developed a new system composed of 3 primers for identify expansion. One of primers was complimentary for CCTG-repeats and for another FAM-marked primer. This enabled to record signal by sequencer. Thus we detected expansion in our patients and confirmed clinical diagnosis.

P12.137 Impact of sequence interruptions on PCR based molecular testing of myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is the most common autosomal dominant neuromuscular disorder of adults associated with unstable expansions of a (CTG)n repeat tract in the 3'-untranslated region of the *DMPK* (Dystrophia Myotonica Protein Kinase) gene. Conventional fluorescent PCR and triplet-repeat primed PCR (TP-PCR) represent widely used methods for detection of normal range and expanded alleles. In our study, we focused on recently described unusual sequence interruptions inside the CTG tract and their effect on these PCR based testing methods. In our assay, both complementary strands of the amplicons produced by conventional fluorescent PCR were labelled with different fluorescent dyes. In addition, TP-PCR was performed in both forward and reverse direction. According to our results, the presence of the unusual (CCGCTG)n containing repeat motifs may lead to mistyping and false results both in conventional and in TP-PCR. In amplifiable alleles using conventional PCR the presence of sequence interruptions may lead to abnormal electrophoretic mobility of one of the complementary strands. In TP-PCR they may lead to discontinuous or even prematurely terminated signal. Since the results obtained by our combination of methods complemented each other, the simultaneous use of bi-directionally labelled conventional PCR and TP-PCR performed in both directions seems to be advantageous for increasing of the reliability and accuracy of the DNA based DM1 diagnostics.

P12.138 The distribution of homozygous deletions of exons 5 of the NAIP gene in subjects with different diseases

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The NAIP gene (5q13.1) is involved in the inhibition of apoptosis and the innate immune response. The alteration of balance between these

processes may represent a component of physiopathology of some common human diseases.

Aim. To test the possible distribution of homozygous deletions of exons 5 of the NAIP gene in patients with obesity, diabetes, breast cancer, colorectal cancer and spinal muscular atrophy.

Subjects: Clinical data and blood samples were collected from unrelated obese subjects (n=150), T1DM (n= 150), T2DM (n=150), breast cancer (n=100), colorectal cancer (n=70) and spinal muscular atrophy patients (n=90). We selected healthy subjects (n=150) with normal BMI (<25 Kg/m²) as a control group.

All samples were investigated for NAIP exon 5 homozygous deletion by duplex PCR.

Results: The homozygous absence of NAIP exon 5 was detected in obese subject (n=2), T1DM (n=2), T2DM (n=3), breast cancer (n=3), colorectal cancer (n=5), spinal muscular atrophy (n=38) and in the control group (n=4). We have observed that the higher frequency of deletion was present in spinal muscular atrophy patients (42.2%), OR = 26.67, 95%CI= 9.0779<O.R.<78.3722, p<0.00001. The incidence of deletion in the control group (2.66%) is higher compared with data reported in other population.

Conclusion: The homozygous deletion of NAIP was similar in all lots, excepting spinal muscular atrophy group, and seems to be at most a weak contributor to investigated pathology with the mentioned exception.

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P12.139 Familial isolated cardiomyopathy caused by a mutation in the flavoprotein subunit of succinate dehydrogenase

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Cardiomyopathies 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. A defect of the human succinate dehydrogenase (SDH) is a rare condition in human, representing 2% of respiratory chain (RC) deficiencies. Its clinical presentation is highly variable, ranging from early onset encephalomyopathies to tumor susceptibility in adults. We present the association of a mutation in the SDHA gene with the clinical manifestation and interfamilial variability of 15 patients diagnosed with dilated and hypertrophic cardiomyopathy. The cardiomyopathy is presumably caused by the significant specific reduction of the SDH enzymatic activity in heart muscle whereas substantial activity is retained in skeletal muscle and lymphoblastoid cells. Identification of the SDHA as the mutated gene was hindered since patients with a similar clinical presentation belonging to the same enlarged family proved to have mutations in another gene and a father homozygous for the mutation is not affected. The same mutation was previously reported to cause a multi-systemic failure leading to neonatal death and to Leigh syndrome. Thus this study highlights the extreme variability that results from homozygosity of the same mutation in a nuclear encoded respiratory complex.

P12.140 Mapping of the neurodegenerative disease PHARC

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PHARC: **Peripheral neuropathy, Hearing loss, Ataxia, Retinitis pigmentosa, and Cataract**, is a progressive autosomal recessive, neurodegenerative disease that was initially ascertained as a phenocopy for Refsum's disease. Recognition of the first symptoms is typically in the teens and cataract, hearing loss and a predominantly demyelinating peripheral neuropathy are present in all adult patients. Retinitis pigmentosa typically starts in young adult life and progresses slowly. The onset and severity of ataxia are variable. Patients with this disease do not manifest abnormalities of peroxisomes, and we embarked on genetic characterisation on the assumption that this would provide new insights into the pathology of such complex neurological syndromes. PHARC was originally mapped to a 16 Mb region on chromosome 20 in a Norwegian family. Subsequently, we have narrowed down the candidate region using SNP-arrays in a further 19 patients from four different countries. In these patients, we have identified four loss of function mutations in a recently characterized gene, two of which represent founder events in Norway and Algeria, respectively.

P12.141 Clinical and genetic study in an Italian family with neurofibromatosis type 1

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Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder with an estimated incidence of 1 in 3,500 births. Clinically, it is characterized by cafe-au-lait (CAL) spots, neurofibromas, freckling of the axillary or inguinal region, Lisch nodules, optic nerve glioma, and bone dysplasias. NF1 is caused from inactivating mutations of the 17q11.2-located *NF1* gene. To date, more than 1000 different germline *NF1* mutations ranging from single nucleotide substitutions to large genomic rearrangements have been identified. We present a clinical and molecular study of an Italian family with Neurofibromatosis type 1. The proband, 10-years-old, showed large cafe-au-lait (CAL) spots and freckling on the axillary region and only a plexiform neurofibromas on the right side. His father, 47 year old, showed in addition to the similar signs, numerous neurofibromas on the thorax, abdomen, back, shoulder of the various size. Other two family's members (a brother and a sister) presented only little cafe-au-lait (CAL) spots. The mutational analysis of the *NF1* gene revealed a novel frameshift insertion mutation in the exon 4c (c.654 ins A) in all affected members of the family. The segregation of the mutation with the affected phenotype and its absence in the 200 normal chromosomes suggest that it is responsible for the NF1 phenotype.

P12.142 Identification of *SPRED1* mutations (Legius syndrome) in patients clinically suspected of having Neurofibromatosis type 1

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Neurofibromatosis type 1 (NF1) [MIM 162200] is a common dominant autosomal disorder, affecting 1 in 3500 individuals, and caused by mutations in the *NF1* gene at 17q11.2. Clinical diagnosis of NF1 requires the presence of at least 2 of 7 criteria defined by the National Institutes of Health (NIH), being the most distinctive features the cafe-au-lait spots and neurofibromas.

Recently, germline loss-of-function mutations in the *SPRED1* gene, located at 15q14, have been related with the Legius syndrome or NF1-like syndrome [MIM 611431]. This condition belongs to a group of disorders with overlapping phenotypes known as neurocardio-facial-cutaneous syndromes, caused by mutations in genes of the RAS signalling cascade. Most of patients with a *SPRED1* mutation fulfil NIH criteria for NF1, although they usually have a more moderate phenotype than those with a *NF1* mutation.

In this study, we screened for mutations of *SPRED1* gene in 145 un-

related patients suspected of having NF1 and which resulted negative for *NF1* mutations. Using direct cDNA sequencing approach, we identified six novel changes: one splice error, two frameshift mutations, two missense changes and a presumed polymorphism.

In conclusion, we strongly recommend molecular analysis of the *SPRED1* gene in patients with clinical criteria of NF1 and without an identified causative *NF1* mutation, in order to increase the sensibility of diagnosis and to offer a specific genetic counselling.

P12.143 Concurrence of germline mutations in the *NF1* and *BRCA1* genes in a family with Neurofibromatosis type 1 and Breast Cancer

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Neurofibromatosis type 1 (NF1) is a common dominant autosomal disorder caused by mutations in the *NF1* gene. Its clinical manifestations and severity are highly variable, even between members of the same family. The main manifestations of NF1 are *café-au-lait* spots, neurofibromas, intertriginous freckling, Lisch nodules, and malignancy, including peripheral nerve sheath tumors, central nervous system gliomas, and a variety of other tumors not so clearly defined. The association between NF1 and breast cancer or other gynaecologic malignancies seems uncommon and has been scarcely referred in the literature. However, a recent population study suggested that NF1 females have a five-fold risk of breast cancer diagnosed <50 years of age.

We have observed a family with two females affected by both NF1 and early-onset breast cancer, and a male with NF1. Taking into account that the family presented criteria of the two hereditary syndromes we evaluated whether the concomitance of both disorders could be attributed to a *NF1* mutation and its supposed increased risk of breast cancer or to the concurrence of two germline mutations, one in the *NF1* gene and the other in a *BRCA1/2* gene. Mutation analyses of *NF1* and *BRCA1* were carried out in one of the females affected by both diseases. We identified a frameshift mutation in *BRCA1* and a nonsense mutation in *NF1*.

Our findings strengthen the importance of considering all phenotypic features in families with both NF1 and malignant tumours, analysing cancer predisposing candidate genes, to offer a specific risk assessment and management of both conditions.

P12.144 High-resolution melting analysis helps to speed up mutation scanning in the *NF1* gene

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Neurofibromatosis type 1 is the most common tumor-predisposing disorder in humans. The large size of the *NF1* gene and the fact that pathogenic changes, mostly point mutations, are dispersed throughout the entire gene with no evidence of mutational hot spots, makes mutation detection quite laborious and costly. In order to speed up mutation scanning and reduce workload and costs we implemented *NF1* analysis by High-resolution melting on a LightCycler® 480. PCR-conditions for amplification of all 60 coding exons including adjacent intronic splicing regions were optimized. Amplification products were checked by PAGE for absence of by-products and sequenced in order to ensure specificity. Validation was performed by testing DNA from 28 patients with mutations previously detected using PCR-SSCP-analysis. Compared to wild-type controls all 28 mutations yielded a clearly distinguishable melting curve visible on a difference plot. In addition heterozygosity for 7 of the most frequent SNPs was also easily detected. Since in the yet limited group of patients tested to date no false negatives were observed, sensitivity was judged to be close to 100% as required for such a test. A small number of false positives were also found. Analysis of a sufficient number of DNA samples in order to estimate the rate of false positive calls is ongoing. In conclusion, High-resolution melting has proven to be a suitable method for rapid and cost effective mutation scanning in the *NF1*-gene.

P12.145 Two *de novo* GC donor splice sites abolishing the authentic site in *NF1* gene in patients with clinical features of Neurofibromatosis type 1

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Accurate pre-mRNA splicing is crucial for gene expression. Splicing involves the recognition of conserved elements at both exon-intron boundaries. The 5' splice site (5'ss) comprises the last three nucleotides of an exon and the first six of an intron, and the 'GU' dinucleotide at the 5' end of introns is almost invariable.

Genomic variants in both coding and non-coding sequences can cause unexpected deleterious splicing defects. Mutations in the neurofibromin 1 (*NF1*) gene, located at 17q11.2, cause Neurofibromatosis type 1 (*NF1*) [MIM 162200], an autosomal dominant disorder affecting 1 in 3500 individuals worldwide. Over the last decade, *NF1* mutation screening strategies at both DNA and RNA level revealed that a significant proportion of mutations affected splicing, even when canonical splicing sequences were unaffected.

Here, we report two novel genomic variants (one translationally silent and one missense) in *NF1* exon 43 in five patients from two unrelated families with clinical features of *NF1*. These mutations create two different *de novo* GC 5'ss, abolishing use of the intact authentic GU 5'ss. The effects of both mutations on splicing in patient cells were recapitulated using minigene constructs transfected into mammalian cells. It is remarkable that two *de novo* GC 5'ss are found within the same exon, because naturally-occurring GC 5'ss are rare (<1%) and there are very few cases of cryptic or *de novo* GC 5'ss.

Our findings highlight the importance of studying mutations at DNA and RNA level in order to clarify their pathological effect and to provide adequate genetic counselling.

P12.146 Two novel mutations in the *NF2* gene in patients with neurofibromatosis type 2.

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Neurofibromatosis type 2 (*NF2*) is a rare dominantly inherited syndrome caused by inactivating mutations in the *NF2* tumour suppressor gene on chromosome 22. Vestibular schwannomas, usually bilateral (BVS), and intracranial meningiomas (IM) occur in 90% and 50% of patients, respectively.

In this study we present 4 cases that have been seen in our hospital in the last year (Table 1)

We report two novel mutations: c.641T>C, p. Leu214Pro, a missense mutation in exon 7 and c.810+1dupG, a splice site mutation in the intron 8 donor site. Missense mutations (case 2) predominantly cause mild phenotypes with an statistically greater survival than nonsense and frameshift mutations. Phenotype is more variable in patients with splice-site mutations (case 4). Furthermore, it is described that carriers of splice site mutations in exons 1-9 are significantly younger at onset of symptoms and have significantly more meningiomas than those with other mutations.

| | Case 1 | Case 2 | Case 3 | Case 4 |
|---|-------------|-----------------------------------|-----------------------------|--|
| Age of onset | 50 | 55 | 45 | 14 |
| Presentation | Imbalance | Hearing loss 25 years ago | Headache | Neck tongue syndrome |
| Finding | BVS | BVS IM: ponto-cerebellar angle | BVS IM (fronto-temporal) | Bilateral VIII and III schwannomas, IM (Meckel cavum, posterior cranial fossa) |
| NF2 molecular study (Sequencing and MLPA) | No mutation | c.641T>C | No mutation | c.810+1dupG |

P12.147 A mild phenotype of autosomal dominant CMT2K disease caused by a new mutation in the *GDAP1* gene

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Hereditary neuropathies are highly heterogeneous group of neurological diseases. The most frequent inherited peripheral neuropathy in humans is Charcot-Marie-Tooth disease (CMT). It can be classified based on clinical, electrophysiology and nerve biopsy morphological analyses. Severity of CMT phenotype is determined by mutated gene and type of mutation. Initially CMT4C4 disease was reported as a hereditary sensory neuropathy inherited as an autosomal recessive trait caused by mutations in the *GDAP1* gene. In 2005 two *GDAP1* mutations (Arg120Trp and Thr157Pro) were reported in CMT families with autosomal dominant trait of inheritance (CMT2K). To date over 30 mutations were identified in the *GDAP1* gene, only 4 of them are inherited in autosomal dominant trait. The authors present a family (3 affected patients), with a new Ala156Gly mutation in the *GDAP1* gene which was transmitted as dominant trait. Phenotype is characterized by a pure axonal sensory-motor neuropathy with a marked involvement of the lower limbs, associated with additional features (cardiac arrhythmia).

We conclude that: (i) CMT2K disease is a mild form of sensory-motor pure axonal neuropathy, (ii) *GDAP1* gene analysis should not be limited only to AR-CMT cases.

P12.148 Molecular diagnostics of neurofibromatosis type 1 (*NF1*) in Slovak patients based on analysis of entire coding region of the *NF1* gene and MLPA

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Neurofibromatosis type 1 (*NF1*; 1:3500) is caused by mutations in RAS negative regulator- neurofibromine 1. Molecular diagnostics requires analyses of the entire *NF1* gene. mRNA-bases screening protocols are preferred since approximately one third of the pathological *NF1* mutations affect mRNA splicing, out of which 30% would remain undetected when only methods based on the analysis of DNA are used. Complex analysis of the *NF1* gene has not been routinely available in Slovakia yet. We introduced and employed diagnostic protocol based on the sequencing analysis of complete coding region of *NF1* as developed by Messiaen and Wimmer (2008), as well as MLPA analysis employing P122, P081 and P082 sets (MRC-Holland). Screening in 37 Slovak *NF1* patients was completed at the time of abstract submission. cDNA sequencing uncovered the presence of small *NF1* mutations in 29 patients: 18 frameshift, 4 missense, 3 stop mutations, 3 typical splicing changes, and one in-frame deletion in exon 39. No mutation was found in 8 patients. Detailed clinical data review showed that 4 of them did not fulfill clinical criteria, thus diagnosis of *NF1* was excluded. MLPA analysis was performed in remaining 4 patients that lack small *NF1* mutations. Type I deletion of the entire *NF1* gene and surrounding region was identified in 2 of them (6,3%), and the intragenic deletion of 5 continuous *NF1* exons was uncovered in one additional patient (3,1%). Thus, our protocol enabled us to identify disease causing mutations in 32 out of 33 clinically confirmed *NF1* patients (97%).

P12.149 NMD-Chip, a European Consortium for next generation genetic diagnostics by DNA arrays

The NMD-Chip Consortium;

INSERM, Marseille, France.

Inherited NeuroMuscular Disorders (NMDs) form a very large and heterogeneous group of genetic diseases, that can be split in Duchenne/Becker (DMD/BMD), limb girdle (LGMD), or congenital muscular dystrophies (CMD), and Charcot-Marie-Tooth neuropathies (CMT). Their overall prevalence is estimated around 1 out of 1000 people.

The current diagnosis is based on a differential molecular genotyping by a complex and time consuming gene by gene approach. As a

consequence, about 30 to 40 % of patients remain devoid of a precise genetic diagnostic.

The aim of the NMD-Chip project is to design, develop and validate high throughput DNA arrays to efficiently diagnose NMD patients. The new tools originating from this project will allow assessing all known genes implied in a group of disease in one shot. Besides, the project will also allow to identify new disease causing mutations by using extensive candidate genes exploration.

Indeed, 4 types of chips are being designed, 2 for known NMD genes, and 2 for candidate genes testing. In each case, both CGH arrays to detect CNV, as well as Sequence Capture for massive re-sequencing (point mutation detection), are set up. We use the new generation of HD2 12-plex chips (Roche-Nimblegen).

The NMD-Chip Consortium comprises 13 European laboratories strongly involved in molecular diagnostics from 8 countries. The project has been funded by the European FP7 Health call for a 2.9 millions of euros, and has started from October the 1st, 2008. The commercial version of the chips should emerge by the end of year 2011.

P12.150 SOS1 mutation analysis in Noonan syndrome and associated isolated congenital heart defects

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Noonan syndrome (NS) is genetically heterogeneous and mutations in the *PTPN11*, *KRAS*, *SOS1*, *RAF1*, *BRAF*, *MEK1*, *SHOC2* and *NRAS* genes have been identified in up to 75% of cases. Here, we screened a large cohort of *PTPN11* and *KRAS* mutation-negative NS patients for *SOS1* mutations and found 6 distinct pathogenic *SOS1* mutations in 26 independent cases, and one rare unclassified variant. Seven *SOS1* mutations were novel. Among the new mutations identified, we observed 5 missense mutations and one small in-frame deletion. Detailed clinical evaluation of *SOS1* mutation-positive subjects confirmed the occurrence of a distinctive phenotype characterized by ectodermal anomalies and congenital heart defects (CHDs), particularly pulmonic valve stenosis and atrial septal defects, with lower prevalence of short stature and cognitive impairment. Two individuals developed tumors. One had mandibular giant cell granuloma (MGCG), while the other had multiple tumors (MGCG, abdominal rhabdomyosarcoma, cerebral glioma and granular cell tumors of the skin), providing further molecular evidence of the linking of these tumors to the RAS-MAPK signaling pathway. Possible involvement of germline *SOS1* mutations in isolated CHD commonly encountered in individuals with *SOS1* mutations (*i.e.*, atrial [23 patients] and ventricular [15 patients] septal defects, and pulmonic valve stenosis [21 patients]) was also explored. None of the patients with isolated CHD was found to carry a functionally relevant change in the entire *SOS1* coding sequence, excluding a major contribution of the gene in the pathogenesis of the investigated CHDs.

P12.151 Identification of a new Jnk-activating familial *SOS1* and a *de novo* *RAF1* mutations in a Noonan syndrome patient

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Noonan syndrome is an autosomal dominant genetic disease characterized by congenital heart defects, short stature and characteristic facial features. Mutations in *PTPN11*, *RAF1*, *SOS1*, *KRAS*, and *NRAS* are responsible for 60-75% of the cases, thus additional genes, are expected to be involved in Noonan syndrome pathogenesis. The genotype/phenotype correlation has been hindered by the relatively few reported genotyped cases. Expanding the case numbers will benefit the clinical community. A mutation analysis has been performed on *RAF1*, *SOS1* and on the *SOS1*-interacting *GRB2*, in twenty-four NS patients previously found to be negative for *PTPN11* and *KRAS* mutations. We identified four mutations in *SOS1* and one in *RAF1*, while no *GRB2* variants have been found. Interestingly the *RAF1* mutation was present in a patient also carrying a newly identified p.R497Q familial *SOS1* mutation, segregating with a typical NS *SOS1* cutaneous phenotype. The new *SOS1* mutations have been predicted to dysregulate the protein activity by bioinformatics. Functional analysis demonstrated the R497Q-*SOS1* mutation leads to Jnk activation, but had no effect on the Ras effector *Erk1*. We propose that this variant might contribute to the onset of the peculiar ectodermal traits displayed by the proband amidst the more classical NS presentation. The characteristic cutaneous traits, frequently shown by NS patients carrying *SOS1* mutations, might be associated to the activation of both Jnk/Erk pathways. To our knowledge, this is the first reported case of a NS patient harbouring mutations in two NS genes, allowing us to propose a genotype/phenotype correlation in the family.

P12.152 A novel missense mutation in the *NDP* gene in a child with Norrie disease

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Norrie disease (ND) MIM 310600 is a X-linked recessive disorder characterized by very early childhood blindness due to degenerative and proliferative changes of the neuroretina. Norrie disease is caused by mutation in the gene encoding norrin (*NDP*; 300658).

We reported three generations Russian Norrie disease family. The proband was a ten years old boy which was normal except for lens opacities found at initial examination at 3 months of age. He had atrophic irides and the fundus was filled with a proliferating retrolental yellowish mass. At 12 months of age, the left eye was enucleated on suspicion of retinoblastoma. Histologic examination showed a hyperplasia of retinal, ciliary, and iris pigment epithelium, hypoplasia and necrosis of the inner layer of the retina, cataract, and phthisis bulbi. CT imaging of the eyes showed small lenses attached to the cornea, opacification of the lenses, corneal opacities. Pars plana vitrectomy and lensectomy was performed.

NDP gene was localized at Xp11.4 and consist of 3 exons, coding exons are exons 2-3. We identified a novel missense mutation Cys-128Trp at exon 3 at proband, his mother and aunt were carriers of this mutation. There were performed two prenatal diagnostics in this family. One fetus was ill male, and the second was healthy female.

P12.153 A novel frameshift mutation at codon 66 (HBB: c.del201A) in the beta-globin genes

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Thalassemias are hereditary anemias. In beta-thalassemia (beta-thal), beta-globin synthesis is either deficient or absent. Smaller, but significant concentrations of beta-thal are present throughout the Middle East, India, Pakistan and China, while sporadic cases have been reported in most ethnic groups. Over 200 beta-thal mutations have been described so far. We report here a novel deletion variant beta-thal allele in an ethnic Korean patient, hitherto unreported in the literature. The deletion spans exon 2 at nt -201 causing a frameshift and the premature appearance of a stop codon. A frameshift mutation in exon 2 of the beta-globin gene [codon 66 (-A)], was associated with a beta-thal phenotype. We report on a 41-year-old woman, of Korean origin, and born out of nonconsanguineous union. She had a history of transfusion dependent anemia since 4 years age. Analysis of her red blood cell parameters and hemoglobin showed microcytic normochromic anemia

with severe anisopoikilocytosis and an elevated unknown Hb band level of 10.4%. This mutation was detected with a polymerase chain reaction (PCR) and direct sequencing method, using a primer designed to detect mutations in total 3 exon. Identification of new mutations will be helpful in better management and counseling for the family regarding prenatal diagnosis in future pregnancies..

P12.154 Consortium for Osteogenesis Imperfecta Mutations in the Helical Region of Type I collagen: analysis of genotype-phenotype relationship using an updated list that doubles the number of previous reported mutations.

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Osteogenesis imperfecta is caused predominantly by mutations in *COL1A1* and *COL1A2*, encoding the pro α 1(I) and pro α 2(I) chains of type I collagen, respectively. The Consortium has assembled 1574 independent mutations, of which 1273 result in glycine substitutions in the triple helical region and 301 alter splice sites. Doubling the number of previously reported mutations (Marini et al. *Hum Mut* 2007) does not alter prior findings that mutations at the N-terminal end of both chains have a non-lethal outcome and that distinct genotype-phenotype relationships can be drawn for each chain. About 40% of glycine substitutions in α 1(I) are lethal and the two exclusively lethal regions (aa.691-823 and 910-964) aligning with Major Ligand Binding Regions (MLBR) remain well defined, suggesting crucial interactions of collagen with extracellular matrix proteins. Currently, 80% of glycine substitutions in α 2(I) are non-lethal. Refining the Regional Model yields 9 lethal clusters and correctly predicts the outcome of 89% of cases in α 2(I). Lethal regions align with proteoglycan binding sites, suggesting a role in fibril-matrix interactions. About 20% of mutations alter splice sites, and are mainly associated with a non-lethal outcome. The Tm of the triplet carboxyl to the mutation predicts over 70% of non-lethal outcomes for Ser substitutions in α 1(I) and Ser and Cys substitutions in α 2(I), but does not apply to lethality predictions. Salt bridge disruption due to Gly substitutions in KGE/D triplets in α 1(I) are associated with lethal outcomes. The expanded mutation database supports different roles for each chain in collagen stability and matrix organization.

P12.155 Absence of Cyclophilin B Causes Recessive Osteogenesis Imperfecta but Does Not Impair Type I Collagen Peptidyl-Prolyl Isomerization

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Osteogenesis imperfecta (OI) is a genetic disorder characterized by bone fragility. Recessive forms of OI are caused by mutations in the three genes coding for the collagen 3-hydroxylation complex. This complex post-translationally hydroxylates α 1(I)Pro986 and residues on types II and V collagen. Null mutations in *CRTAP* or *LEPRE1* lead to severe/lethal forms of OI with white sclerae and rhizomelia. Fibroblast collagen has minimal Pro986 hydroxylation, but is overmodified, indicating delay in collagen folding. In Senegalese siblings, we identified a homozygous mutation in the start codon of *PPIB*, which encodes cyclophilin B (CyPB) the third member of the 3-hydroxylation complex. These children have moderate OI and white sclerae, but not rhizomelia. Proband fibroblast RNA had 55% of normal *PPIB* transcripts. On Western blot, no CyPB protein was detectable, while both *CRTAP* and *P3H1* levels were reduced. Immunofluorescence microscopy confirmed these findings *in vivo*. CyPB is a peptidyl-prolyl *cis-trans* isomerase and thought to be involved in the rate-limiting step of type I collagen folding. Surprisingly, proband collagen was not overmodified and had normal levels of lysyl and 4-prolyl hydroxylation, in contrast to collagen overmodification by cells with recessive defects in *CRTAP* or *LEPRE1*, or with *PPIB* mutations which lead to misfolded CyPB. Pro986 hydroxylation was also normal in proband collagen, indicating that the complex can 3-hydroxylate collagen in absence of CyPB. Therefore, complete absence of CyPB does not appear to delay collagen folding, strongly suggesting a redundancy for collagen isomerization, or CyPB may not be the major type I collagen folding isomerase.

P12.156 PARK2 mutations are rare among Polish patients with Parkinson disease.

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Parkinson disease (PD) is the most common neurodegenerative disorder, affecting more than 1% of the population above the age of 60. Mutations in the *PARK2* gene result in early-onset PD (EO-PD). The frequency of the *PARK2* mutations as a cause of EO-PD is a matter of a debate now. According to the early studies up to 18% of EO sporadic PD carry *PARK2* mutations. In familial cases this frequency was up to 50%. The last years' studies show the data overestimation. For the Polish EO-PD patients the frequency of *PARK2* mutations in both alleles was estimated at the level of about 2.5%.

The aim of presented study was to analyse the frequency/type of changes in *PARK2* gene in PD patients.

The group of patients consisted 160 subjects (104 EO-PD, 56 LO-PD), mainly sporadic but also familial. Mutation analysis of all *PARK2* exons was performed by direct sequencing and dosage analysis.

The detected variability in *PARK2* in - 3.1% alleles (without coding polymorphisms p.S167N, p.V380L, p.D394N, p.R402C) included exon deletion and duplication (Ex4,5,6,7 del, Ex2 dup, Ex 2,3,4,5 dup) as well as point mutations (c.203_204 AG del/p.Q34Xfs, c.346C>A/p.A82E, c.478_479 CAC ins/p.P133dup, c.734A>T/p.K211N, c.924C>T/p.R275W). Only c.203_204AG del was a recurrent mutation (frequency 0.94%), the remaining represented single cases (0.31%).

Mutations of two alleles of the *PARK2* gene were identified only in 3 EO-PD patients - 1 familial (AR inheritance) and 2 sporadic cases. Heterozygous mutation carriers were identified among EO and LO-PD patients (Ex2dup and p.R275W and p.A82E, c.478_479 CAC/p.P133dup respectively).

P12.157 Whole Exome Sequencing in Parkinson patients

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Genetic factors have been identified as risk factors for the development of Parkinson's disease (PD). Overall these genes only explain a minority of PD cases. It has recently become possible to sequence all coding exons in one single experiment. PD is a late onset complex disease with several known pathways being central to the disease. Here we used whole exome sequencing as the most suitable approach to identify novel high penetrance PD genes.

Genomic DNA was enriched by NimbleGen on array and Agilent in solution enrichment. Sequencing was performed on the SOLiD platform. Each sample was sequenced on four Quads generating a minimum of 60 million 50 base pair reads per exome.

With the Agilent protocol 97.73% of targeted exons were represented by reads (versus 89.89% with NimbleGen). In total, 95% of reads were on target using Agilent versus 65% of reads for NimbleGen. NimbleGen enrichment is designed for the 454 platform which might explain off target reads. Using an end depletion protocol to remove NimbleGen adaptors the percentage of reads on target did not improve. Overall, 95% of targeted exons were covered 10X with a maximum coverage reaching 1500. With sufficient coverage for SNP calling, approximately 60000 variants were identified per patient. In total, 5234 variants within a 20 basepair window of exons could not be found in dbSNP and HapMap databases. We are in the process of analysing these SNPs with respect to potential pathogenicity.

P12.158

Molecular analysis of the parkin and PINK-1 genes in patients with early-onset Parkinson disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder where genetic as well as environmental factors are implicated in its etiology.

Mutations in the *parkin* and *PINK1* (PTEN-induced putative kinase) genes have been identified as the most common causes of autosomal recessive early-onset Parkinson disease (EOPD).

We performed the mutational screening in thirty-four clinic patients with a mean age: 40.2 years, including both familial (n=6) and sporadic (n=28) cases for *parkin* and *PINK1* mutations.

Over sporadic cases, we found five variants in *parkin* gene: homozygous deletion of exon 3 was found in one patient and homozygous missense mutation (Cys212Tyr) in another index case. A compound heterozygote genotype was detected (Del154A in exon 2 and Thr415Asn in exon 11) and two heterozygous carriers with deletion of exon 6 and a missense mutation in exon 3, respectively.

We detected a heterozygous individual with a nonsense mutation (Gln456X) in *PINK1* gene.

All of variants were previously reported as aetiology of the disease. No mutations were found in patients with a positive familial history. Mutations in DJ-1 (Park 7) are also associated with EOPD but are less frequent.

In conclusion, mutations in the studied genes account for 17.64% of our casuistic. We show a wide variety of different mutations that are associated with EOPD.

Interestingly, in three early onset cases we have detected only one mutation with putative dominant effect which suggests that heterozygous cases of *parkin* and *PINK1* genes could be penetrant depending on the type of mutation.

P12.159 LRRK2 mutation analysis in a cohort of Parkinson's Disease patients with a dominant pattern of inheritance from Southern Italy

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Mutations in the LRRK2 gene (locus PARK8) have been associated both in PD patients with a dominant pattern of inheritance and patients which appear to be sporadic. In most populations studied, LRRK2 gene mutation frequencies range from 1.7 to 13% among familial cases, and from 0.4 to 2.7% in sporadic cases. Reported frequencies may be lower limits because only particular exons or mutations were screened in most studies. Although many variations have been identified in LRRK2, assessment of association with the PD phenotype has not been clear-cut because of incomplete penetrance and lack of segregation data for many of the familial cases. Mutations affecting

residues p.G2019 in exon 41 and p.R1441 in the exon 31 are common confirmed disease-associated variations. The frequencies of these mutations are highly population dependent as well as the putative risk factor variations R1628P (exon 34) and G2385R (exon 48).

We hereby present the preliminary results of mutation screening of exons 31, 34, 35, 38, and 48 of LRRK2 gene in 80 autosomal dominant PD patients from Southern Italy. Mutation analysis was performed by PCR and sequencing. Our cohort was negative for alpha-synuclein gene mutations and LRRK2 exon 41 mutations (p.G2019S, p.I2012T and p.I2020T). Until now we screened 35 patients for these five LRRK2 exon. We identified three described polymorphisms (IVS47-9 del(T), A7155G in exon 48; IVS35+23T→A intr 36) but no mutations in these patients. Preliminary data showed a low mutation frequency of LRRK2 gene in our Southern Italy PD cohort.

P12.160 Exon 41 LRRK2 point mutation analysis in Italian patients with Parkinson disease.

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Mutations in *LRRK2* gene (OMIM 607060) have been found in a large number of Parkinson's disease patients. *LRRK2* encodes a large protein of 2527 amino acids with multiple domains, including a non-receptor tyrosine kinase-like domain, a Ras-like small guanidine triphosphatase family domain (Roc), and several ankyrin, leucine-rich, and WD40 repeats. Several mutations in different functional regions of *LRRK2* occur at a high frequency both in familial and sporadic PD (sPD). Of these, the p.Gly2019Ser mutation laying in exon 41 has been reported to account for 5-6% of familial PD and 1.6% of sPD in Europe.

In this study we screened *LRRK2* exon 41 by DHPLC and/or direct sequencing to examine whether p.Gly2019Ser, the most common point mutation, was present in our cohort of 1348 PD patients with different ages of onset. All samples derived from the „Human genetic Bank of patients affected by Parkinson disease and parkinsonisms“ (<http://parkinson.it/dnabank.htm>) Istituti Clinici di Perfezionamento, Milan-Italy. A total of 42 patients were found to carry exon 41 *LRRK2* mutations/variants/polymorphisms:

34 p.Gly2019Ser heterozygotes

1 p.Gly2019Ser homozygote

1 p.Gly2019Ser - IVS41+30 A>G compound heterozygote

1 p.Ile2020Leu heterozygote

1 p.Pro2036Arg heterozygote

1 p.Tyr2018Tyr heterozygote

1 p.Pro2007Pro heterozygote

2 IVS40-39 A>G heterozygotes

According to previous European studies we found that the most frequent amino acid change in the *LRRK2* gene is the p.Gly2019Ser. Meanwhile other aminoacid changes have also been identified. We speculate that some of them may also be pathogenic for the *LRRK2* protein kinase activity.

P12.161 Influence of paraoxonase 1 status on age at onset and progression of LRRK2-linked Parkinson's disease.

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Mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene are the most frequent cause of the familial Parkinson's disease (PD). The variability in age at disease onset (AAO) and neuropathology in *LRRK2*-linked PD patients suggests that genetic and environmental factors may influence the course of the disease other than mutation itself. Paraoxonase 1 (PON1) is a detoxifying enzyme of human's blood plasma which protects from the influence of potential neurotoxins (organophosphates such as pesticides, etc.). The 54M PON1 allele variant and the reduced activity of paraoxonase 1 were associated with an increased risk of PD. We studied the influence of PON1 status (L54M

PON1 polymorphism, activity of the enzyme) on AAO and disease progression of *LRRK2*-linked PD. Eleven *LRRK2*-linked patients (8 patients patients with G2019S mutation, 2 - V1613A, 1 patient - R1441C; average age 67.6±10.3), patients with sporadic PD (sPD) (average age 70.6±5.3) and individuals of control group (average age 67.1±8.2) were taken into analyses. L54M *PON1* genotyping was carried out by PCR with the following restriction digestion analysis. Paraoxonase 1 activity was measured comparatively to the paraoxon substrate on the spectrophotometer "SmartSmecPlus". We didn't reveal any significant differences between studied groups ($p>0.05$) (Table 1). Thus, the paraoxonase 1 status doesn't influence the development of *LRRK2*-associated PD.

| | LRRK2-linked PD AAO<60 years | LRRK2-linked PD AAO≥60 years | LRRK2-linked PD with rapid progression | LRRK2-linked PD with slow progression | sPD (n=15) | Controls (n=15) |
|-------------------------------------|------------------------------------|------------------------------------|--|---|---------------|--------------------|
| Frequency of 54M <i>PON1</i> allele | 0.50 (n=5) | 0.58(n=6) | 0.33 (n=3) | 0.63 (n=8) | 0.33 | 0.40 |
| Paraoxonase 1 activity | 27.89±15.91 (n=3) | 25.67±11.86 (n=4) | 43.50 (n=1) | 23.78±11.00 (n=6) | 29.19±9.09 | 29.32±9.85 |

P12.162 To facilitate automatic variant pathogenicity assessment gene variant databases need to provide structured general disease information

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Gene sequence variant databases (Locus-Specific DataBases, LSDBs) store DNA variants in genes involved in hereditary disease, the way they were detected and by whom (a reference). Details on the associated clinical phenotype and the potential disease-causing effect (pathogenicity) are rare or absent. Recently, prediction of the pathogenic effect of sequence variants by computational means has become feasible. Clinicians and researchers working in DNA diagnostics check LSDBs frequently to classify and evaluate the effect of a specific variant in a particular carrier. Most LSDBs provide links to OMIM disease information, but not much information about the mechanism causing a particular disease (disease etiology). As a consequence insufficient disease information is presented to allow correct interpretation of the pathogenic effect of variants and this may lead to incorrect interpretation of individual risks. This may cause distress in patients and their families accessing variant data in web-based databases. In addition it prevents large-scale automatic pathogenicity assessment of variants identified by genome-wide re-sequencing. We propose that LSDBs add general disease information to their homepages to support correct individual risk prediction by computational means. For each associated phenotype, the minimal information necessary for risk predictions includes a.o: inheritance pattern, locus heterogeneity, imprinting and known additional genetic and environmental factors involved in disease etiology. In the near future, these details should be accessible through webservices for automated analysis by specific reasoners. Funded in part by the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 200754 - the GEN2PHEN project.

P12.163 Mutation analysis of the gene *PMP22* in a cohort of Slovak patients with Charcot-Marie-Tooth disease and hereditary neuropathy with liability to pressure palsies

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The aim of our study was to identify the spectrum of mutations and their frequencies in Slovak patients with Charcot-Marie-Tooth disease type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP), which are the two most prevalent inherited peripheral neuropathies. Both of them are caused by distinct mutations of a single gene encoding peripheral myelin protein 22 (*PMP22*), duplication and deletion of the whole gene being the most frequent underlying mutations. The rest of CMT1A and HNPP patients harbour point mutations

in the same gene.

In the present study we screened 121 unrelated families indicated with unspecified CMT and 2 unrelated families indicated with HNPP for causative mutations in *PMP22*. We detected the CMT1A duplication in 41 families, whereas the HNPP deletion was present in 8 families of which 7 were originally diagnosed as CMT. Further screening identified one family with an already described point mutation in exon 5, namely c.327C>A (Cys109X). Our results suggest that the spectrum and frequency of mutations in Slovak patients with CMT and HNPP is similar to that seen in the global ethnic population. Altogether, mutation analysis of the *PMP22* gene revealed the underlying cause of disease in 40,65% of examined families.

P12.164 The coexistence of the *PMP22* gene deletion and the *EGR2* gene mutation in background of patients with CMT 1a hereditary sensomotor neuropathy

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Background: A number of genetic defects may cause hereditary neuropathies. The most frequent form of HMSN is CMT disease. The demyelinating form of CMT1a is caused by a 1.5MB duplication of the *PMP22* gene in 70% of the cases. The genetic defect of the *EGR2* gene may result in also autosomal dominant neuropathy.

Methods: Above a thorough clinical examination, ENG, EMG, sural nerve biopsy and genetic testing has also been performed. Quantification of the *PMP22* gene was carried out by Real-Time PCR. The coding exons of the *MPZ* and *EGR2* genes were sequenced using the ABI Prims 3100 sequencing machine. The obtained sequence was compared with the human reference sequence.

Results: The first symptoms of the 44 and 48 year old brothers appeared in their early twenties with distal type muscle atrophy and paresis. ENG showed severe demyelinating polyneuropathy. Segmental demyelination could be seen in the sural nerve biopsy of the younger patient. Genetic testing found a deletion of the *PMP22* gene in the older brother, who had more severe symptoms. In both brother a pathogen substitution c. 1142 G>A (Arg381His) was revealed in exon 4 of the *EGR2* gene.

Discussion: It is the first report about the coexistence of *PMP22* gene deletion and a pathogen mutation of the *EGR2*. The coexisting 2 mutations did not aggravate the clinical symptoms. Previously the c. 1142 G>A (Arg381His) mutation was shown to be associated with cranial nerve involvement, which was not present in our patient.

P12.165 Study of Peutz- Jeghers syndrome patients in Poland

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Peutz-Jeghers syndrome (PJS) is rare, genetically conditioned disease. PJS is herited in autosomal dominant manner and is characterized by occurrence of hamartomatous polyps. The hamartomatous polyps are manifested during second or third decade of life. The polyps can be located throughout digestive tract. Occurrence hamartomatous polyps in PJS may cause of many gastrointestinal discomforts. Although in PJS patients the risk of malignant transformation is lower then others hereditary neoplastic disease, an increased risk to development malignancies such as the pancreas, the breast, female and male reproductive organs is observed. The second characteristic manifestations of PJS are brown, dark or blue spots. PJS is caused by mutations in the *LKB1* (STK11) on chromosome 19. *LKB1* gene encodes a serine/threonine protein kinase participating in very important cell signaling pathways. The PJS diagnosis of 20 patients was based on presence of two or more polyps, or one polyp and typical pigmented lesions, or one polyp and a family history of PJS. Mutations screen-

ing analysis revealed seven mutations and one polymorphism. These mutations are located in different position in gene. With the Multiplex Ligation-dependent Probe Amplification (MLPA) - assay we detected additional genomic mutations. For our screening we used the SALSA P101 STK11 kit which contains MLPA probes for most STK11 exons. In seven patients we identified exonic deletions or duplications range from one to five exons.

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P12.166 The mutational analysis of the *PAH* gene in families with phenylketonuria

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Phenylketonuria (PKU) is a common autosomal recessive genetic disorder caused by a large variety of mutations in the phenylalanine hydroxylase gene (*PAH*).

We have carried out the mutational analysis of the *PAH* gene in 174 families with PKU from Bashkortostan Republic, North Caucasus and Kazakhstan. Consequently we revealed 32 various mutations that spectrum was specific for each region. Our results indicated that *R408W* mutation accounted for 54.27% PKU in Bashkortostan and 42.5% in Kazakhstan. Mutation *R261X* of the *PAH* gene is found out only in patients from the North Caucasus with frequency of 38.16% whereas mutation *R408W* in the given region is revealed with frequency of 13.16 %. In addition we identified mutations *R261Q*, *c.1315+1g>a*, *c.1066-11g>a*, *R158Q*, *R252W* and *P281L* of the *PAH* gene with frequencies from 9.77 to 1.72 % on the average. Using SSCP analysis followed by sequencing of the *PAH* gene we have detected 19 mutations with frequencies from 1.72 to 0.29%: *c.663-664delAG*, *S349P*, *L48S*, *c.441+5g>t*, *c.47-48delCT*, *Y414C*, *c.208-210del3bp*, *E390G*, *A300S*, *P211T*, *R413P*, *c.168+5g>a*, *R243X*, *R243Q*, *E280K*, *c.1089delG*, *D415N*, *c.509+5delg* and *R111X*. Six new mutations were revealed as well: *R252P*, *c.116delT*, *Y206X* (*c.618TAC> TAA*), *c.1315+del4* in PKU patients from Bashkortostan, *F331S* - from North Caucasus and *S350Y* - from Kazakhstan.

80.5% of the studied families with PKU have appeared completely informative for direct DNA-diagnoses, 17.8% - partially-informative, and 1.7% - absolutely not informative.

Thus the data of mutational analysis may be used for prenatal diagnostics and carrier screening in PKU families.

P12.167 X-linked hypophosphatemic rickets: mutational analysis of *PHEX* gene in an Italian cohort

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Phosphate (P) plays a key role in bone and mineral metabolism. Among various causes of hypophosphatemia are inherited disorders of P homeostasis, including X-linked hypophosphatemia (XLH), autosomal dominant hypophosphatemic rickets (ADHR), and autosomal recessive hypophosphatemia (ARHR). Affected patients show similar clinical features, such as short stature, bowing of the legs, and signs of rickets, and typical biochemical findings (hypophosphatemia, reduced $TmPO_4/GFR$, high-normal PTH with low or normal $1,25(OH)_2D$ concentrations).

XLH, ADHR and ARHR are caused by mutations in *PHEX*, *FGF23* and *DMP1* genes, respectively.

We describe a cohort of 22 pedigrees with clinical, biochemical and radiological diagnosis of HR: 9 were familial cases and 12 were sporadic cases. Using DHPLC (denaturing high-performance liquid chromatography), MLPA (multiplex ligation-dependent probe amplification) and sequence analysis, we screened all affected individuals for mutations in *PHEX* gene: 21 mutations were identified (95%), 14 of which are novel. The mutations include 5 missense (23.8%) and 5 nonsense mutations (23.8%), 3 small deletions (14.3%), 2 small insertions (9.5%), 3 splice-site mutations (14.3%), and 3 gross deletions (14.3%). One patient did not have a *PHEX* mutation. She was subjected to mutational analysis of both the untranslated regions (5' UTR and 3' UTR) of *PHEX*, as well as was screened for *FGF23* and *DMP1* genes muta-

tions; no mutation was found in those regions.

Genetic testing is useful to confirm the diagnosis of HR, to identify mild forms of HR in family members, and for appropriate genetic counselling. Our data indicate that there is no single predominant *PHEX* mutation XLH.

P12.168 Molecular studies of the *PANK2* gene in patients with PKAN

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Pantothenate kinase-associated neurodegeneration (PKAN) is an autosomal recessive disorder characterized by progressive dystonia, rigidity, choreoathetosis, spasticity, retinitis pigmentosa, optic atrophy, parkinsonism and iron accumulation in the brain. Clinical data suggest two forms of PKAN: a classic form characterized by early onset and rapid progression and an atypical form with later onset and a more slowly progressive course. Many patients with classical and atypical PKAN have mutations in the gene encoding pantothenate kinase 2 (*PANK2*) and a specific magnetic resonance imaging (MRI) pattern called eye-of-the-tiger.

In this study we performed a mutational analysis of the *PANK2* gene in 10 PKAN patients from Italy and France. Brain MRI examinations were not available for all the patients. The entire coding region (seven exons) was investigated for point mutations by sequencing analysis; furthermore, we used PCR real time to identify any possible exonic rearrangements. In 9 patients no *PANK2* mutations were identified; only one patient showed an already described point mutation, but in the heterozygous state.

Our results excluded *PANK2* point mutations and exonic rearrangements in our patients, both in atypical and classic forms. This confirms the genetic heterogeneity in PKAN and therefore the importance of investigating the role of other responsible genes.

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P12.169 Mutations in the *PKHD1* gene at autosomal recessive polycystic kidney disease in patients from Russian Federation.

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Autosomal recessive polycystic kidney disease (ARPKD, MIM 263200) is a severe disorder with variable clinical spectrum. It is an important cause of renal-related and liver-related morbidity and mortality. 30-50% of affected babies die shortly after birth in respiratory insufficiency because of pulmonary hypoplasia. Due to the poor prognosis there is a strong demand for prenatal diagnosis. ARPKD gene was mapped to chromosome 6p21 and prenatal diagnosis was performed with polymorphic microsatellite markers from this region. But sometimes material from the dead baby was not available. That is why very important to looking for gene mutations in materials of the parents. *PKHD1* gene (MIM 606702) has the longest open reading frame that is encoded by a 67-exon transcript. Mutations were found to be scattered throughout the gene without evidence of clustering.

For DNA analysis we select 11 exons where were found according to literature more than one mutation in more than one family. We analyzed DNA samples of babies and parents from 49 families. We found 12 mutations on 28 chromosomes for 21 families. There were identified 6 previously unknown mutations: *G130A*, *C1427A*, *3797delC*, *G5908A*, *A8864G*, *10585_10588delGAAT* and 6 previously described mutations. There were no mutations identified in 28 families. It could be because of genetic heterogeneity or assumed diagnosis of ARPKD might be incorrect in some cases. Mutation analysis in *PKHD1* gene is very important for the confirmation of the ARPKD diagnosis and for the genetic consultation with following prenatal diagnosis.

P12.170 The Study of PAH Gene (Classic PKU) in Iranian Patients

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Phenylalanine hydroxylase (PAH) deficiency results in intolerance to the dietary intake of the essential amino acid phenylalanine and produces a spectrum of disorders including phenylketonuria (PKU), non-PKU hyperphenylalaninemia (non-PKU HPA), and variant PKU. Classic PKU is caused by a complete or near-complete deficiency of phenylalanine hydroxylase activity; without dietary restriction of phenylalanine, most children with PKU develop profound and irreversible mental retardation.

PAH deficiency is inherited in an autosomal recessive manner. At conception, each sib of an affected individual has a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier. Prenatal diagnosis of PAH deficiency is possible in pregnancies at 25% risk either when molecular genetic testing has revealed the disease-causing mutations in the PAH gene in an affected family member, or when linkage analysis has identified informative markers.

The PAH gene, located on chromosome 12q22-24, consists of 13 exons and spans 90 kilobase.

Our laboratory uses PCR-based sequencing technology to identify mutations among the 13 exons. This method identifies approximately 99% of mutations.

30 patients investigated for PAH in this study .6 mutations were fund; 969+5 G>A in 3 patients , 1089 del G in 2 patients , 782 G>A in 2 patients , 843-5 T>C in 1 patient , 168+5 G>C in 1 patient , 441+1 G>C in 1 patient.

P12.171 Screening for phosphomannomutase deficiency in samples of foetuses and newborn infants with congenital malformations

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Phosphomannomutase (PMM2) deficiency is the most frequent glycosylation disorder affecting the N-glycosylation. The clinical manifestation spans from severe hydrops fetalis, fetal loss and multisystemic disorders and central nervous system involvement in infancy. The aim of this work was to investigate the presence of PMM2 deficiency in a cohort of 228 foetal samples and 230 dried blood samples from newborns, all with congenital malformations. We have identified ten samples with PMM2 changes in heterozygosity. We have identified four previously described nucleotide changes (p.R141H, p.R123Q, p.C241S and p.T237R) and three novel ones (p.R238H, p.F157S and IVS3-5T>C). The most frequent disease-causing mutation p.R141H in the PMM2 gene was identified in four independent alleles (4/460 alleles). This fact was not surprising according to the high allelic frequency reported in the general population (1/79). The rest of the variant changes were identified in only one allele each. In addition, no aberrant transcripts or deletions have been detected using whole genome SNP array (Illumina610K) and transcriptional profiling analysis in two samples bearing p.R141H and p.R238H, respectively. Expression analysis in a prokaryotic expression system of the changes p.R123Q, p.F157S, p.C241S and p.R238H showed 1%, 0%, 60% and 100% PMM 2 residual activity, respectively. These results suggest that all the changes detected in the PMM2 gene are disease-causing mutations with the exception of p.R238H that might be a non-synonymous SNP. These findings are clearly in disequilibrium with the reported frequency of PMM2-CDG-Ia and require further genetic and functional analysis to provide more insight about these interesting results.

P12.172 Molecular investigation of PMP22 gene in Russia CMT1 patients: comparison of different methods

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Charcot-Marie-Tooth (CMT) disease, also known as hereditary motor and sensory neuropathy, is among the most frequent hereditary disorders of the nervous system.

225 unrelated patients with clinical and electrophysiological data of CMT1 were observed by us. In the first time the most common mutation - duplication at the chromosome 17p11.2-12 locus - was investigated by polymorphic (CA)n repeats that flanking PMP22 gene. At 121 patients duplication was deduced. Next time multiplex ligation-dependent probe analysis (MLPA) was carried out for all patients without mutations. We created two systems: the first - using probes were designed to evaluate all PMP22 coding exons and four control genes: *TBP*, *SIRT3*, *USP3* and *B2M*, the second - using probes were designed to evaluate first, second, third and fifth PMP22 exons and four control genes: *TBP*, *SIRT3*, *USP3* and *B2M*. At three patients deletion of the second PMP22 exon was deduced and at one patient the disease-cause tetraplication of 17p11.2-12 region was detected. In the absence direct sequence analysis of the all PMP22 exons 1-5, including their intron/exon boundaries was undertaken. At one patient Leu147Arg mutation was detected.

Accordingly the part of CMT1A compose 56% in all cases of CMT in Russia and the necessity of use complex access at the diagnostics of CMT1A was shown.

P12.173 Molecular and cytogenetic investigations of patients affected with premature ovarian failure.

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Premature ovarian failure (POF) is a heterogeneous disorder, defined also as premature menopause or hypergonadotropic ovarian insufficiency. Clinically, it is manifested by the secondary amenorrhea for at least four months in women under 40 years of age with elevated level of follicle-stimulating hormone FSH > 40 IU/l and decreased level of estradiol E2 < 20 IU/L. Investigations involving cytogenetic tests have shown that POF occurrence may be associated with aberrations localized mainly in the long arm of chromosome X or premutation in FMR1 gene.

The material used in this study comprised 40 DNA samples from patients with clinical symptoms of POF.

The cytogenetic analysis of the material revealed two X/autosome translocations, whereas DNA analysis showed FMR1 gene premutation in three patients. The frequency of X/autosome translocation in this material was 2/40 (5.0%) and FMR1 gene premutation was 3/40 (7.5%). Overall the applied genetic tests allowed the identification of the cause of POF in 5 per 40 cases (12.5%).

P12.174 Identification of a chromosomal loci associated with recessive Primary Ciliary Dyskinesia in a Bedouin family

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Primary ciliary dyskinesia is a rare genetic disorder, autosomal recessive, 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 the 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. Numerous healthy siblings making the family suitable for positional cloning of the affected genes.

Linkage to the 13 known genes associated with the disease was negated.

Genome wide linkage analysis using the Affymetrix GeneChip map-

ping 250K array was performed on two patients and their parents from the family.

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. Prioritizing genes for search of the mutation and initial sequencing, was preformed according to 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 novel techniques to diagnose subjects with PCD.

P12.175 Identification and characterization by MLPA and aCGH of a whole PROP1 deletion in a girl with pituitary mass and combined pituitary hormone deficiency

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Introduction: Mutations in at least five genes encoding pituitary specific transcription factors (PROP1, POU1F1, LHX3, LHX4, HESX1) result in combined pituitary hormone deficiency (CPHD), characterized by proportionate growth deficit, due to impaired production of growth hormone and one or more of the other five pituitary hormones. Recessive mutations in PROP1 are the most frequent defect detected in CPHD. The girl, born to a consanguineous relationship, first presented at the endocrinology clinic at the age of 8.8 yr with growth failure (height 108.8 cm, -3.48 SDS). She was diagnosed with CPHD after hormonal evaluation. MRI of the pituitary gland showed a suprasellar mass. She did not have diabetes insipidus, neuro-ophthalmologic complaints or visual fields abnormalities. We decided to undertake PROP1 mutation screening.

Methods: PROP1 mutation screening included MLPA (Salsa P216, MRC Holland) and array CGH of chromosome 5 (Nimblegen).

Results: A homozygous deletion of the entire PROP1 was detected in the girl. Both parents were shown to be heterozygous for this deletion. The deletion was delimited to at least 7.3kb upstream of PROP1 and more finely to ~907bp downstream from the stop codon.

Conclusion: We describe the third CPHD case with a complete PROP1 deletion in homozygosity. The 5' breakpoint appears to lie in a highly repetitive region, rich in Alu sequences. The 3' breakpoint lies within an AluSx repeat suggesting that the deletion may have arisen through non-allelic homologous recombination. In the presence of CPHD and pituitary mass, PROP1 analyses should be considered before referring the patient to a neurosurgeon.

P12.176 Nevoid basal carcinoma syndrome (Gorlin syndrome) and pronounced androgenic alopecia in a female with a novel mutation p.Leu 1159fsX32 of the PTCH gene.

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Nevoid basal cell carcinoma syndrome(NBCCS) or Basal cell nevus syndrome (BCNS)(OMIM109400) or Gorlin syndrome is an autosomal dominant disorder with complete penetrance. Prevalence varies from 1:57,000-1:256,000 with no sexual predisposition. *PTCH1* gene has been mapped to the long arm of chromosome 9(q22.3-q31), and consists of 23 exons (34kB) encoding a transmembrane protein. Data suggests that *PTCH1* gene functions both as a tumor-suppressor gene and as a developmental regulator of normal tissues. More than 284 different type mutations have been reported in patients with NBCCS with over 80% resulting in truncation of the coded protein and haplo-insufficiency. We report a 55-year-old woman with multiple(>80) BCCs all over her body, mostly of the nodular type, appeared as done-shaped papules with telangiectatic surface and a pearly translucent border,

some of which were crusted or ulcerated and firm. A heterozygous c.3475delC mutation in exon 21 of *PTCH* was found. To the best of our knowledge, this is a novel mutation, which due to its truncating nature, it is most likely disease-causing. Outstandingly, she presented also pronounced androgenic alopecia. It is not currently known whether *PTCH* plays a role in male pattern alopecia but *PTCH* protein is involved in Sonic hedgehog (Shh) signaling required for the initiation of anagen phase of the hair follicles. Thus, we postulate that epidermal cells lacking normal *PTCH* function may exhibit a defect in responding to Shh during the hair cycle. This is the first case in the relative literature of pronounced androgenic alopecia in female patient with Gorlin syndrome.

P12.177 R408W mutation among PKU patients of Leningrad province

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Phenylketonuria (PKU) is an autosomal recessive disorder arising from the deficiency of some enzymes which catalyze the essential conversion of Phe to Tyr. In the majority of cases PKU is caused by the mutations in the phenylalanine hydroxylase (PAH) gene. More than 500 mutations have been described worldwide. The incidence of this inborn error of metabolism in the population of Leningrad province was estimated to be 1:6400 - 1:10266. R408W is one of the most frequent mutation in St. Petersburg population. In present study we have identified the mutant allele R408W in 57 patients revealed according to the data of neonatal screening in Leningrad province. Identification of R408W allele in the dry spot samples was done with PCR method. 21 probands were found to be homozygous (R408W/R408W). 36 probands were shown to be compound with R408W as one mutant allele. There were 68,4% of R408W allele among chromosomes investigated

P12.178 Homozygosity Mapping in Consanguineous Spanish Families with Autosomal Recessive Retinitis Pigmentosa

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Purpose: To identify the genetic defect in Spanish pedigrees affected by autosomal recessive retinitis pigmentosa (arRP).

Methods: A total of 14 patients from five consanguineous Spanish families with typical RP was included in this study. After exclusion of the known mutations using the arRP and LCA genotyping microarray chips (Asper Biotech), whole-genome SNP genotyping combined with homozygosity mapping was performed to find the causative genetic defect. In significant homozygous regions, microsatellite markers were used to confirm and narrow down the linkage intervals. Direct sequencing of candidate genes in these regions was performed to find the causative mutations.

Results: In two out of five families studied (RP0055 and RP0285), homozygous regions encompassing the *EYS* gene were identified. Direct sequencing of *EYS* revealed a homozygous c.5928-2A>G mutation in all affected members of the RP0055 family, producing an alteration in the splice site. In family RP0509, the largest and second largest homozygous regions included *ABCA4* and *CNGB1* respectively. For the RP1190 family the two largest regions overlapped with the *RP22* locus and *CNGB1* whereas for family RP0371, homozygosity mapping and microsatellites analysis revealed a novel locus with a significant LOD Score (2.12).

Discussion: Homozygosity mapping is a useful tool as a first step to identify the genetic cause in Spanish RP families. In four out of five families studied, our results suggested that mutations in known RP genes are implicated in this retinal disorder. We present the first splicing defect in *EYS* and furthermore identified a novel arRP locus.

P12.179 Identification of two novel CDKL5 mutations in Hungarian patients with Rett syndrome phenotype

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In our study we screened 159 individuals for MECP2 mutations with Rett phenotype. A total of 22 different known mutations were identified in 40 subjects: 8 frameshift-deletions, 4 nonsense mutations, 10 missense mutations. Among the pathologic mutations the most frequent were the Arg133Cys (9.8%), Thr158Met (9.8%), Arg255Stop (7.8%), and Arg294Stop (7.8%) changes. We also detected the missense C925T (Arg309Trp) mutation in an affected patient; however, the role of this alteration in Rett pathogenesis is still unknown according to mutation databases. As a unique variant, we detected an inherited 18bp deletion, 1162_1179del18 in a patient who carried the frameshift-associated mutation of 276insG.

In all patients (n=108) having no MECP2 defects detectable by direct sequencing, we screened for mutations of CDKL5, a serine-threonine kinase gene recently identified in patients with Rett phenotype. We discovered two novel nonsense mutations: G607T that results in a premature termination codon at amino acid position 203 disrupting the catalytic domain of the CDKL5 protein, and G1708T leading to a premature stop at amino acid position 570 of the C-terminal region involved in either the catalytic activity or the subcellular localization. Our results suggest the need of screening for CDKL5 mutations in patients with Rett phenotype tested negative for MECP2 mutations.

P12.180 Mutational analysis of the MECP2 gene in Tunisian patients with Rett syndrome: a novel double-mutation

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Rett syndrome is a severe disorder characterized by loss of acquired skills after a period of normal development in infant girls. Purposeful hand use is often lost and replaced by repetitive, stereotypic movements. This X-linked dominant disorder is caused mainly by mutations in the MECP2 gene. In this report, we performed a mutational analysis of the MECP2 gene in 7 Tunisian patients with classical Rett syndrome. The results showed the presence of a double-mutation: p.R306C and the c.1461+98insA which create a new hypothetical polyadenylation site in the 3'UTR of the MECP2 gene. We also detected a new variant c.1461+92C>G in the 3'UTR located previous to 34 bp from the polyadenylation site with a score of 4.085. This variation is located in a hypothetical splicing enhancer with a score of 1.96277 according to the ESE finder program. We also found 2 common mutations: p.T158M (57.14 %) and p.R168X (14.28 %).

P12.181 RFX6 mutation in a newborn with congenital diabetes and hemochromatosis**

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A newborn from consanguineous parents was referred prenatally for intrauterine growth retardation and anomalies of the digestive tract. The prenatal work up included an *in utero* MRI, which showed abnormalities consistent with hemochromatosis, intestinal atresia, and gallbladder aplasia. These diagnoses were confirmed postnatally. Diabetes was present since birth. Interestingly, both parents, and many

of their first- and second-degree relatives had diabetes or glucose intolerance. This infant contributed to the identification of the RFX6 winged helix transcription factor as the cause of the Riley-Mitchell syndrome of digestive tract malformation and congenital diabetes, by a strategy of homozygosity mapping and high-throughput sequencing. In early stages of mouse development, RFX6 is broadly expressed in the endoderm, and is restricted to the pancreas and some gut foci in later stages. In the adult it is essentially expressed in the pancreatic islets. The infant we report here was homozygous for the Arg181Gln mutation, which abolished binding of RFX6 to its cognate DNA site. It is unclear to what extent the Riley-Mitchell syndrome differs from the Martinez-Frias syndrome. Our observation suggests that congenital hemochromatosis might be a feature of the RFX6 defect.

P12.182 The RIN2 syndrome: a new autosomal recessive connective tissue disorder caused by deficiency of Ras and Rab interactor 2 (RIN2)**

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In 2005 we reported a new recessive EDS-like syndrome with fleshy swelling of facial tissues and severe scoliosis in a consanguineous Algerian family with three affected siblings. Recently, a homozygous 1-bp deletion in the RIN2 gene, encoding the Ras and Rab interactor 2, was shown to be involved in a similar phenotype, termed MACS (Macrocephaly, Alopecia, Cutis laxa and Scoliosis) syndrome. Important phenotypic overlap between our EDS-like family and the MACS family prompted us to re-assess our family and perform molecular analysis of RIN2.

The most striking clinical features included progressive facial dysmorphism and (kypho)scoliosis, sparse hair as well as skin- and joint hypermobility. Ultrastructural studies of the skin revealed important abnormalities in the collagen fibril morphology. Fibroblasts exhibited a dilated endoplasmic reticulum and an abnormal Golgi apparatus with rarefied and dilated cisternae. Molecular analysis of RIN2 identified a novel homozygous 2-bp deletion in all affected individuals. The c.1914_1915delGC mutation introduces a frameshift and creates a premature termination codon. The resulting aberrant mRNA is prone to nonsense-mediated mRNA decay, probably resulting in loss-of-function of the corresponding protein.

Our findings show that RIN2 defects are associated with a distinct autosomal recessive genodermatosis of which the progressive facial coarsening, gingival hypertrophy and scoliosis are the most striking features. Although, the current family displays considerable phenotypic overlap with MACS syndrome, the skin phenotype belongs to the Ehlers-Danlos, rather than the cutis laxa spectrum. This study underscores the involvement of RIN2 and associated intracellular trafficking pathways in the pathogenesis of heritable connective tissue disorders.

P12.183 Molecular investigation for Rett Syndrome in female patients with severe mental retardation.

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Rett syndrome (RTT) is an X-linked dominant neurodevelopmental disorder affecting mostly girls. RTT is characterized by normal early development, followed by psychomotor regression and gradual onset of microcephaly. Most RTT cases (70-95%) are caused by mutations in MECP2 gene, while recently another gene, CDKL5 has also been correlated with the RTT phenotype. This study reports on the incidence and spectrum of MECP2 gene alterations, in female patients referred for variable phenotypes within the RTT spectrum. During the last five years, 159 girls - of which only 35 completed the RTT scoring system- underwent molecular investigation for severe developmental delay/ mental retardation (MR). Of those, 56 requested analysis for RTT (group 1) and 103 analysis for RTT and Angelman syndrome

(AS) (group 2). Molecular analysis with ECMA (Enzymatic Cleavage Mismatch Analysis) and direct sequencing of exons 3 and 4 of the MECP2 gene was performed in 135 subjects allowing detection and characterization of disease causing mutations. RTT was confirmed in 41/159 subjects [29/56 (group 1) and 12/103 (group 2)] where common mutations, novel alterations and previously reported disease related polymorphisms were detected. Silent polymorphisms were also revealed and remain under investigation. Three patients carried alterations of the CDKL5 gene and were also classified as having RTT. In addition, alterations of the 15q11-13 region were disclosed in three girls classified as AS. MECP2 gene alterations should be considered as a cause of MR. Clinical evaluation using the RTT scoring system would improve the diagnosis and research of RTT especially in respect to phenotype-genotype correlation.

P12.184 A rare un-explained case of Sandhoff disease among Iranian population

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Sandhoff disease is a rare, genetic, lipid storage disorder resulting in progressive neurodegenerative disorder and caused by a deficiency of the enzyme beta-hexosaminidase (HEX), which results in the accumulation of GM2 gangliosides in the brain and other organs of the body. Sandhoff disease is a severe form of Tay-Sachs disease. Weakness begins in the first 6 months of life. Inheritance pattern is autosomal recessive with multiple alleles and compounds. Mutations occur in HEXB, encoding the β -subunit, cause the neurodegenerative condition, Sandhoff disease. This gene contains 14 coding exons that located on chromosome 5q13. The proband was a one year old boy with progressive muscle weakness and psychomotor retardation. Biochemistry analyses of hexosaminidase A and B showed HEXB deficiency in the patient. This case admitted with muscle weakness, seizures, myoclonus, visual impairment and cherry-red macular spots. Molecular assessment revealed a homozygote missense mutation „K 121 R“ in exon 2 of this gene and his parents showed a heterozygote state in this region. In spite of the Banerjee report (1991) such a mutation converted „K 121 R“, as a polymorphism since it also occurs in some normal subjects, our finding from whole gene sequencing suggest that this mutation can cause the disease in homozygote condition. However, more functional study is needed to clarify this molecular aspect of the disease.

P12.185 Identification of seven novel HGSNAT mutations in eleven Sanfilippo C patients: characterization at the enzyme activity and RNA levels, and analysis of the origin of frequent mutations

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Mucopolysaccharidosis III (MPS III), or Sanfilippo syndrome, include four autosomal recessive diseases characterized by a deficient heparan sulphate degradation. Clinical symptoms are similar for all types of MPS III, including progressive and severe deterioration of the central nervous system during childhood. HGSNAT is the gene responsible of MPS IIIc, which encodes the acetyl CoA: α -glucosaminide N-acetyltransferase, a lysosomal membrane protein.

In this study we have identified all the mutant alleles in eleven patients, seven Spanish, three Moroccan and one Argentinean. Nine different changes have been found. Seven of them were novel: two splice-site mutations (c.633+1G>A and c.1378-1G>A), four missense mutations (c.161C>T, c.338T>C, c.1271G>T and c.1334T>C, and one 19-nucleotide intronic deletion. The deleterious effect of latter was demonstrated by a minigene assay. Splicing defects were all confirmed at the RNA level. Heterologous expression analyses in COS-7 cells of HGSNAT cDNAs bearing missense mutations showed negligible enzyme activity for all of them.

The two most frequent mutations, c.234+1G>A and c.372-2A>G, had been previously described. Mutation c.234+1G>A was always found in a

double-mutant allele with p.P237Q, as described for Moroccan patients. Interestingly, we found p.P237Q in 3 alleles from 118 Moroccan healthy individuals. These data, together with expression results, confirm that it is a low frequent polymorphism. A haplotype analysis was performed using a novel polymorphism and 14 previously described SNPs. The results are consistent with a single origin for each one of these two frequent mutations. Mutation c.234+1G>A seems to have a common origin, due to founder effect, in Moroccan and Spanish patients.

P12.186 Several SCA17 minor expanded CAG/CAA alleles with incomplete penetrance and variable expression coexist with other SCA1 and SCA3 expansions.

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Spinocerebellar ataxia type 17 (SCA17) is an autosomal dominant neurodegenerative disease characterized principally by ataxia, pyramidal and extrapyramidal signs, cognitive impairments, psychosis and seizures. It is caused by a CAG repeat expansion in the TATA-box binding protein gene (*TBP* gene) (6q27), which is translated into a polyglutamine tract. The disease threshold goes from 43 to > 60 CAG/CAA repeats, with reduced penetrance in lower alleles (43 to 48). In one pedigree we found one 65 years female carrying a 44 CAG/CAA allele but without any symptoms of the disease. Surprisingly, also has an allele of 44 repeats in SCA3 locus (14q24), in pre-mutation and low penetrance range. She has transmitted the SCA17 expansion twice to their offspring: a daughter has inherited an expanded allele of 44 repeats and suffers disartria, dementia and cerebellum atrophy; and a son also with 44 repeats but minor psychiatric symptoms and without cognitive impairments or ataxia. We present also four SCA17 affected cases with 43 CAG/CAA repeats with several particular clinical features as ataxia, vertigo, axonal neuropathy and in one case camptocormia (to our knowledge the first description of this symptom associated with an expanded allele in the *TBP* gene). Interestingly, two of these individuals show also expanded alleles from SCA1 (6p23) (48 CAG) and SCA3 (41 CAG). Our results show the eventual penetrance of minor SCA17 alleles of 43 repeats, the great clinical variability expression in SCA17 affected cases and the unexplained coexistences between SCA17 and other SCA repeat expansions as SCA1 and SCA3.

P12.187 SCA8 and other SCA or FRDA expansions coexist in members of several pedigrees with ataxia.

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Spinocerebellar ataxias (SCAs) and Friedreich's ataxia (FRDA) are a group of neurodegenerative diseases in which several genes have been cloned: SCAs 1-3, SCAs 6-7, SCA12 & SCA17 with dominant inheritance; and *FXN* (frataxin) for FRDA, a recessive disorder. In SCAs the mutation usually consists in a CAG or CTG (for SCA8) triple nucleotide repeat expansions, which normally localize in exonic regions and codifies for a polyglutamine tracts of respective proteins. In FRDA the mutation is a triple nucleotide GAA great expansion in the first intron of *FXN* gene. In a Spanish sample of N = 166 unrelated index cases, 6.02% were SCA1; 26.51% SCA2; 34.94% SCA3; 7.23% SCA6; 6.02% SCA7; 15.06% SCA8; 1.20% SCA17; and 3.01% DRPLA. We present here five pedigrees in which SCA8 coexists with other SCA expansions in affected index cases or in relatives. In three pedigrees SCA8 expansions coexist respectively with SCA2, SCA3 and FRDA in the respective index cases. In relatives of each mentioned families the expansions segregates independently one from each other, as we could expect between loci belonging to different chromosomes. Other family shows SCA8 and SCA1 expansions in several members, but none joint both. The same occurs in a pedigree with SCA6 and SCA8. More unexplained SCA8 coexistences have been reported, involving other neurodegenerative disorders as Parkinson or Alzheimer's diseases. These coexistences could be due to high population prevalence

of SCA8 non penetrant expanded alleles of moderate size. More evidences should be addressed to propose alternative hypothesis about cause - effect relationship.

P12.188 Mutation spectrum in the *SMARCB1* and *NF2* genes of sporadic and familial Schwannomatosis patients.

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Schwannomatosis is a genetic disorder which is characterized by multiple peripheral nerve schwannomas and, in contrast to NF2, absence of VIII cranial nerve tumors. Schwannomatosis patients carry mutations in the *SMARCB1* or *NF2* genes, of which the former has been reported as the main casual factor of Schwannomatosis.

Here we report the results of comprehensive *SMARCB1* mutation screening in sporadic and familial Schwannomatosis cases. We identified a group of 18/55 patients, including four familial cases, who had germline mutations in the *SMARCB1* gene (one nonsense, two missense, two duplications, four frameshift and nine splice site mutations). We have also identified a cohort of 37/55 patients (characterized by absence of vestibular schwannomas by MRI and presence of multiple schwannomas) with no germline mutation in the *SMARCB1* and *NF2* gene, including 6 familial cases.

In 15/55 patients, we analyzed at least 1 tumor sample besides the peripheral blood (a total of 22 distinct tumor lesions were analyzed from 15 patients). We found *SMARCB1* or *NF2* mutations in 14/22 tumors. Deletions encompassing both *SMARCB1* and *NF2* genes were the most prominent change in the tumors.

Our results strengthen the hypothesis that there is another, as yet unidentified factor behind the pathogenesis of Schwannomatosis. This casual factor might be shared between Schwannomatosis and AT/RT phenotypes as cases of both disorders were diagnosed in different relatives in one of the families from our study.

Arkadiusz Piotrowski and Andrzej Poplawski contributed equally.

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P12.189 Molecular analysis of the *SCN1A* gene in patients with Severe Myoclonic Epilepsy of Infancy

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Severe myoclonic epilepsy of infancy (SMEI), or Dravet syndrome, is a severe autosomal dominant epileptic encephalopathy mainly caused by de novo mutations in the voltage-gated sodium channel gene (*SCN1A*). Most of these mutations are nonsense or frameshift; missense mutations are also common. Recent studies have already reported that about 12% of mutation-negative SMEI patients have microchromosomal abnormalities involving *SCN1A*. However, the rates of detection of *SCN1A* mutation in SMEI patients range widely in the different populations. To investigate the frequency of *SCN1A* mutations in our population, we analyzed 19 SMEI patients (10 males and 9 females) from Southern Italy, who fulfilled the strict clinical definition of SMEI. Mutation analysis was performed by direct sequencing; the genomic anomalies were screened using multiplex ligation-dependent probe amplification (MLPA). Six different heterozygous coding variants were found in 6 unrelated SMEI cases (31.5%): 4 novel mutations (Thr1289Ile, 3840insT, IVS7+4delA, Glu1021X) and 2 previously described (IVS24-2A/G, Ser1505X). Using MLPA assay we identified a deletion of exons 1-25 in one of the 13 patients without *SCN1A* point mutations (8%). The 3840insT and IVS24-2A/G mutations were de novo; the parents of the other patients were not available. The frequency of *SCN1A* mutations is lower in our SMEI patients in comparison to other populations. Our results confirm the importance of screening the coding regions with both direct sequencing and a quantitative method and suggest that further genetic studies are needed to determine the causative mutations and genes involved in the remaining *SCN1A*-negative patients.

P12.190 Characterization of four familial *SHOX* duplications by MLPA

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SHOX duplications limited PAR1 region appear to be very rare and the presence of long range transcriptional enhancers (ECS) located 3' and possibly 5' of the *SHOX* gene makes complicated phenotype-genotype correlations and the clinical significance of the only four cases report is unclear (Thomas et al, 2009). That study concluded that only larger duplications including the transcriptional enhancers represent a potential cause in cases of idiopathic tall stature.

In our study we describe the clinical and MLPA characterization of four familial duplications (table 1). MLPA analysis was carried out using SALSA P018D1 *SHOX* MLPA (MRC Holland), which includes probes for each exon of *SHOX* and promoter and regulation regions.

All duplications are between, at least 50kb and 460kb, and contain all the *SHOX* coding sequence but different amounts of flanking sequence (table 1). In two cases (family 3 and 4) duplication encompasses several ECS including two shown to have transcriptional activity, but in only one case is associated with tall stature - macrosomy (family 4). All duplications were transmitted from one of their parents.

These results do not seem to support an association between the presence of transcriptional ECS in duplicated region and tall stature, nor identify any potential phenotypic consequences of *SHOX* duplications. Anyway, it would be interesting to perform another specific analysis with these cases (more probes, microsatellites, aCGH) to specify size of duplications.

Table 1:

| Cases: | Phenotype / Karyotype | Subtelom (MLPA) | Size of <i>SHOX</i> duplication. PAR1 region map(scale kb) |
|----------|--|--------------------|--|
| Family 1 | Speech delay and macrosomy (8 yrs. W: >p95 H: >p95 and OFC: 1,3 SD). 46,XY | P036/070 Dup X/Yp | Duplication >=50 kb (MLPA P018C) |
| Family 2 | Short stature (2 yrs. W, H and OFC <p3). 46,XX | | Duplication between. 390-680 kb (MLPA P018-D1) |
| Family 3 | Speech delay and learning disabilities (7 years. W: p50, H: p75 and OFC: 2SD). 46,XY | P036: Dup X/Yp | Two duplications: first between 185-500kb and second between 560-695 kb (MLPA P018-D1) |
| Family 4 | Psychomotor retardation and macrosomy (14 mths. W: p75-90, H: p97 and OFC >2SD). 46, XY | P036/070: Dup X/Yp | Duplication between 460-730 kb (MLPA P018-D1) |

P12.191 PAR1 deletion/duplication in patients with dyschondrosteosis or idiopathic short stature

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Mutations or deletions affecting production of the short stature homeobox-containing gene (*SHOX*) are found in subjects ranging from isolated short stature (ISS) to Léri-Weill (dyschondrosteosis), and Langer syndromes.

Our sample was made of 42 unrelated probands in charge of the Department of Medical Genetics (GTH and 1stFM, Charles University in Prague). Eleven of them were diagnosed as patients with dyschondrosteosis and 30 of them as ISS. All probands were analysed using the MLPA kit P018 that covers PAR1 pseudoautosomal region (including *SHOX* gene) and neighbouring X specific sequences. Overall, ten unique PAR1 rearrangements were detected, eight in the group with dyschondrosteosis (73%), and two in the ISS group (7%). As for the dyschondrosteosis group, seven deletions were indicated as causal what was not the case of found duplication outside the *SHOX* gene. In the ISS group, one causal deletion spanning *SHOX* gene, and one duplication outside the *SHOX* gene, with ambiguous effect, was observed. In addition, a frequent small deletion was traced in one subject with dyschondrosteosis (9%) and in seven individuals with ISS (23%). Our study indicates that small PAR1 rearrangements are quite frequent in Czech population.

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P12.192 A case of homozygous sickle cell disease in a patient from Senegal.

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A patient from Senegal was referred to our center with suspected sickle cell disease; he was in good general health conditions and never transfused. The haematological data showed an increased rate of HbF. We confirmed the homozygosity for HbS with Reverse Dot Blot (RDB) for the β globin gene. The clinical manifestation were not in agreement with molecular result so we analysed the α and γ globin genes. The α -RDB showed the -4,2 deletion in the α gene and sequencing analysis an heterozygous point mutation in the promoter of γ^G gene (-158 C>T). We confirmed this mutational framework by familiar analysis to define the reproductive risk of the parents.

The father genotype was heterozygous both for HbS and the γ mutation but in addition he presented two defects in the α gene (- 4.2 deletion and triplication of α genes aaa). The mother was heterozygous for the HbS and the γ^G mutation and negative regarding α gene. The sister was heterozygous for HbS and the γ^G mutation. She also inherited the aaa from the father. The brother presented only the aaa.

We can now fully explain the phenotype of the proband by the presence of the α and γ defects. In addition we defined the reproductive risk of the family: for the parents in case of an homozygous HbS fetus with co-inheritance of the aaa; for the sister and the brother, in case of a β carrier partner of having an affected fetus with respectively severe or mild clinical manifestation.

P12.193 Multilocus hypomethylation in Silver-Russell syndrome: does tissue-specific distribution of epigenetic mosaicism explain the phenotype?

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Aberrant methylation or mutations at specific loci are common findings in all known congenital imprinting disorders. Interestingly, recent studies revealed that patients with transient neonatal diabetes mellitus and Beckwith-Wiedemann syndrome (BWS) may carry multilocus hypomethylation (MLH). Additionally single BWS and Silver-Russell syndrome (SRS) patients have been reported carrying the same MLH pattern in blood lymphocytes affecting paternally as well as maternally imprinted genes. However, the reason why the same MLH patterns may either result in BWS or SRS is currently unclear.

We now report on the molecular findings in blood and buccal swab DNA in three SRS patients with hypomethylation of both imprinting center regions (ICRs) in 11p15.

Whereas this aberrant methylation affected both ICRs in leukocytes, in buccal swab DNA of two patients only the ICR1 hypomethylation was visible. One of these patients had a healthy monozygotic twin who also carried ICR1 and ICR2 hypomethylation in leukocytes but not in buccal swab DNA. A third patient showed loss of methylation of both ICRs in 11p15 but also of the MEST locus on chromosome 7, this aberrant pattern could also be detected in buccal epithelium.

Screening of several factors involved in establishment and maintenance of methylation marks including ZFP57 did not reveal the molecular clue for the MLH in our patients. However, our data provide evidence that in case of MLH the epimutation which is predominant in tissues others than blood is causative for the phenotype and therefore explains the clinical outcome.

P12.194 No evidence for hemochromatosis type 4 in hemizygous SLC40A1 deletion carriers

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The SLC40A1 gene product, a protein called ferroportin 1, plays an essential role in the regulation of iron levels in the body. Several heterozygous mutations in the SLC40A1 gene have been described to date and were found to cause an autosomal dominant form of iron overload, known as hemochromatosis type 4 (HFE4) or ferroportin disease. The family described in our study carries the complete loss of one allele of the SLC40A1 gene due to a hemizygous deletion, leading to a-priori true haploinsufficiency. Blood samples from four members of this family (three deletion carriers and one non-carrier as a control subject) were available for genetic and biochemical investigations. Thorough analysis of blood parameters revealed that none of the deletion carriers (15-year-old female, 18-year-old male, and 49-year-old male) developed hyperferritinemia. This finding is in contrast to the situation with heterozygous SLC40A1 mutations, which have been reported to cause an early increase in serum ferritin. Our study is the first description of a hemizygous deletion of the entire SLC40A1 gene (true haploinsufficiency) and the corresponding normal phenotype, extending the molecular aetiology of HFE4. Potential mechanisms leading to the identified lack of association of SLC40A1 deletion/haploinsufficiency and HFE4 are discussed.

P12.195 Genotyping the survival motoneuron genes by an high-performance single-base extension: carriers and SMA affected people identification plus prognostic evaluation of SMN2 copy number and SNPs.

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By combining the basis of quantitative genotyping assay and the guiding principles of quantitative real-time PCR we developed a sensitive method with large dynamic range and the potential for high-throughput accurate quantification of allele-specific copy number and SNPs recognition.

We apply this method to the spinal muscular atrophy (SMA) carriers and affected people identification as well as to the allelic and SNP quantification of the disease modifier SMN2 gene. SMA is a severe neuromuscular disease characterized by degeneration of alpha motor neurons in the spinal cord, which results in progressive muscle weakness and paralysis. The vast majority of SMA cases have a childhood onset and different disease severity and course being subdivided in different clinical groups. The primary SMA-determining gene is the SMN1 gene that in about 95% of affected patients is absent independent of the type of SMA. A nearly identical gene, indicated as SMN2, can vary the allelic copy number per genome and has been shown to decrease the severity of SMA phenotype in a dose-dependent manner. Furthermore, a single base substitution (c859G>C), recently identified in SMN2 gene, seems to act as a further positive modifier of SMA phenotype. Our new genotyping method will be applied to an appropriate cohort of patients, in a phenotype-genotype association study, to evaluate the diagnostic and prognostic potential of SMN2 copy number variation and single base substitution.

P12.196 Detection of heterozygous SMN1 deletions in SMA families using a simple fluorescent multiplex PCR method

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With a prevalence of 1/6000 live births, spinal muscular atrophy (SMA) represents the second most common fatal autosomal recessive disorder. The heterozygote frequency has been estimated to be 1/40. The SMA locus has been mapped to chromosome 5q11.2-q13.3 within a region characterized by the large inverted duplication of a 500 kb element. However, the duplication of the SMA locus makes the detection of SMA carriers in the general population difficult, and this has

hampered genetic counseling in affected families. Initial attempts to estimate the SMN copy number were based on the measurement of the SMN1/SMN2 ratios, but the broad variability of SMN2 copy number hinders reliable quantification. In the present study, we describe a method, which allows easy detection of heterozygous SMN1 deletions in SMA carriers and SMA patients without homozygous SMN1 deletions. We simultaneously amplified exon 7 of the SMN1 and SMN2 genes using a mismatch primer X7-Dra, which introduced a *Dra*I restriction site into amplified SMN1 exon 7 and RB exon 13 genes as a control fragment with a two copies of the gene. Digestion samples were analyzed by capillary electrophoresis on ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The size of the peak is determined by measuring its peak area. Given that there are two copies of the RB gene, the relationship between SMN1/RB is used to determine the relative number of copies of SMN1-gene. Using this method, we found heterozygous deletion of exon 7 and/or 8 of 50 analyzed parents of children with SMA.

P12.197 Effect of copy number variation of SMN1 neighboring genes on SMA phenotype in Tunisian patients

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder with an estimated incidence of 1 in 10,000 live births. SMA is characterized by destruction of a motor neurons of anterior horn cells of spinal cord, which leads to muscle weakness and atrophy. This disease is characterized by a high clinical variability. It has been classified into four types (type I: severe, type II: intermediate, type III: mild and type IV: adult). In 95% of cases, SMA is related to homozygous deletion of exon 7 of SMN1 gene localized on 5q13.

The main objective of this study was to determine a genotype-phenotype correlation between the copy number of SMN2, NAIP, p44, H4F5 and *occludin* genes localized in the same region and the severity of the disease in SMA Tunisian patients. Twenty six unrelated patients affected by SMA were enrolled in the study. MLPA and QMPSF were used to measure copy numbers of these genes.

Our results showed that 31.3 % of type I patients carried one copy of SMN2, while all patients of other forms had at least 2 copies. NAIP was absent in 87.5% of type I patients. Furthermore, all SMA type I patients had one copy of H4F5. No correlation was found for p44 and *occludin*. We conclude that there is a close relationship between SMN2, NAIP and H4F5 gene copy numbers and SMA disease severity, which is compatible with the previous reports.

P12.198 The assessment of influence of 5q13 locus genes copy-number on the severity of spinal muscular atrophy in Russian patients.

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Proximal spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disorder caused by the loss of a-motor neurons in the spinal cord. SMA patients are subdivided into types I-III according to age of onset and achieved motor abilities. All three forms of proximal SMA are caused by mutations in SMN1 gene. About 95% of SMA cases are caused by homozygous deletion of the SMN1 gene or conversion events. The phenotype variability of the disease with such molecular homogeneity can be explained by presence of phenotype modifiers. In this study SMN1 and NAIP gene-copy number has been analyzed for establishing of the phenotype-genotype correlation in 130 SMA patients with homozygous deletion of SMN1 gene (SMA0 n=44, SMAII n=43, SMAIII n=43). Thereto, it had been developed a quantitative assay based on Multiplex Ligation-dependent Probe Amplification. Also, we studied phenotype-genotype correlation within the one type of SMA. SMA patients were subdivided into two groups: SMA0 (inherent, diminished fetal movements in utero, with asphyxia and severe weakness at birth) and SMAI (the classical form of severe spinal muscular atrophy). Type III SMA was subdivided into two groups: SMAIIIA (onset below 3 years of age) and SMAIIIB (onset at the age \geq 3 years).

(Tab.1) These results confirmed the lower copy number of SMN1 gene, the higher the severity of disease.

| SMN1 gene-copy number | | Number of SMN1 gene copies and the size of deletion in SMA region | | | | |
|-----------------------|----------------|---|-----|-----|-----|----------------------|
| | | n=2 | n=3 | n=4 | n=5 | Absence of NAIP gene |
| SMAI | SMA0 (n=20) | 90% | 10% | | | 45% |
| | SMAI (n=24) | 58% | 33% | 9% | | 17% |
| SMAII (n=43) | | 56% | 40% | 4% | | 7% |
| | SMAIIIA (n=12) | 50% | 42% | 8% | | |
| SMAIII | SMAIIIB (n=31) | 39% | 29% | 29% | 3% | |

P12.199 Expanding SCN2A-associated phenotypes from neonatal epilepsy to episodic ataxia, myoclonus and pain

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Inherited and *de novo* mutations in sodium channel genes underlie a variety of epilepsy phenotypes. Mutations in SCN2A, encoding the brain sodium channel $\text{Na}_v1.2$, have previously been reported to be associated with benign familial neonatal-infantile seizures, febrile seizures plus and intractable epilepsy of infancy. We evaluated the clinical characteristics in a patient with a neonatal-onset complex episodic neurological phenotype. We screened SCN2A for mutations and carried out *in vitro* electrophysiological analyses to study the consequences of the identified mutation. We studied the developmental expression of $\text{Na}_v1.2$ in cerebellum by immunohistochemical analysis. The patient presented with neonatal-onset seizures and variable episodes of ataxia, myoclonia, headache and back pain after 18 months of age. The patient carries a *de novo* missense mutation (p.Ala263Val) in SCN2A, which leads to a pronounced gain-of-function, in particular an increased persistent Na^+ current. Immunohistochemical studies suggest a developmentally increasing expression of $\text{Na}_v1.2$ in granule cell axons projecting to Purkinje neurons. These results can explain a neuronal hyperexcitability resulting in seizures and other episodic symptoms extending the spectrum of SCN2A-associated phenotypes. The developmentally increasing expression of $\text{Na}_v1.2$ in cerebellum may be responsible for the later onset of episodic ataxia.

P12.200 A 134 kb duplication 0.5 Mb upstream of SOX9 associated with hermaphroditism in an XX male and autism in his XY brother

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In a sibship of six a 46,XX male hermaphrodite with ovotestes and no detectable SRY sequence had a brother with infantile autism without mental retardation. Both brothers had inherited an identical 134 Kb duplication of the regulatory genomic region 0.5 Mb upstream of SOX9 from their mother. In another brother who had inherited the same maternal SOX9 haplotype, no upstream duplication was found, proving that the mother was a gonadal mosaic for the duplication. We hypothesize that the duplication caused sufficient SRY-independent SOX9 expression in the undifferentiated fetal gonads to promote testicular development. Apparently, this effect was tissue- and/or fetal-specific as no SOX9 expression was detectable in leukocytes and skin fibroblasts from any of the siblings. The observations in the family also suggest that such a SOX9 regulatory region duplication may predispose for autism in the presence of a Y chromosome, i.e. a SRY gene. This hypothesis is supported by the SOX9 segregation pattern which as a less than 8,2 % probability of being a random event, and the unexplained general excess of autistic features in males. We therefore propose that an additional role of SOX9, directly linked to testicular differentiation, could be the promotion of male-specific brain development.

P12.201 Validation of 7500Dx (Applied Biosystems) real time PCR instrument for the identification of spinal muscular atrophy (SMA) healthy carriers.

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder caused by degeneration of spinal alpha-motor neurons. On the basis of clinical severity, three forms of infantile SMA can be identified (type I-III). All forms of SMA share the same genetic defect: about 95% of patients have the homozygous absence of SMN1 gene, due to deletion or gene conversion. SMA is a relatively common condition, being the prevalence about 1/6000 and the frequency of healthy carriers around 1/36. Due to the frequency of this condition, the identification of carriers among relatives of patients is a common request to genetic clinics. A number of molecular assays for carrier testing have been developed so far, based on real time PCR or multiplex ligation-dependent probe amplification assay (MLPA). In our Centre we have developed an assay based on real time PCR for SMA carrier testing by which we have analyzed almost 1000 individuals, using 7900 HT Fast Real Time PCR System (Applied Biosystems). Recently, in a collaborative project with Applied Biosystems we have validated the 7500Dx instrument for SMA carrier testing. To this aim, DNA samples from 50 consecutive individuals, relatives of SMA patients or their partners, afferent to the Genetics Clinic at the Catholic University Hospital, were analyzed both by 7900HT and 7500Dx instruments. The results were concordant among the two instruments in 100% of samples analyzed. Our data indicate that 7500Dx instrument is a powerful tool for the identification of SMA healthy carriers.

P12.202 Spectrum of phenotypes associated with the SMN2 c.859G>C variant in Spanish SMA patients.

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by loss or deficiency of the telomeric copy of the *Survival of Motor Neuron* (SMN) gene, *SMN1*. A second, highly similar centromeric gene (*SMN2*) can only partially compensate for *SMN1* deficiency because exon 7 is skipped from most *SMN2* transcripts due to a single C-to-T transition, which results in a partially defective, unstable protein. Recent reports have indicated that the rare c.859G>C nucleotide exchange in *SMN2* is a positive modifier of SMA; patients with only two *SMN2* copies present an unexpectedly mild phenotype, which is related to the enhanced inclusion of exon 7 in transcripts of the *SMN2* variant. We analyzed the prevalence of the c.859G>C base change in a cohort of 253 unrelated Spanish SMA patients and in six pairs of SMA discordant siblings; all patients had homozygous absence of the *SMN1* gene. We identified the gene variant in 10 unrelated SMA patients. In contrast to previously reported cases, we find that c.859G>C is associated with a wide spectrum of phenotypes from typical type II patients who can only sit to adult walkers with type IIIb. Further, all 10 patients share common alleles with multicopy markers linked to SMN genes, strongly suggesting that the variant originated from a common ancestor. Structural data support the view that the variant reduces affinity for the splicing repressor hnRNP A1, favouring the inclusion of exon 7 and increasing the amount of functional SMN protein.

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P12.203 Quantification of *SMN2* gene copy number for molecular diagnostics in Russian SMA patients

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Spinal muscular atrophy (SMA) is neuromuscular disorder caused by homozygous deletion of *SMN1* gene in more than 90% patients. *SMN2* gene copy number, a nearly identical centromeric copy gene of *SMN1*, correlates with SMA severity because *SMN2* gene produces 10-20% of full-length SMN mRNA and functional SMN protein. Clinical trials of

drugs that increase full-length SMN mRNA production by *SMN2* gene are in progress. *SMN2* gene copy number seems to be useful molecular marker for SMA diagnostics.

SMN2 gene dosage analysis was performed for 61 patients affected by II and III types of SMA (42 and 19 patients accordingly) by means of real-time quantitative PCR. Most of II type patients had 3 *SMN2* copies (76.19%), 11.90% of patients had 2 *SMN2* copies and 11.90% had 4 *SMN2* copies, none of them showed one copy. Most of III type patients possess 3 *SMN2* copies (57.89%), 36.84% of patients had 4 *SMN2* copies and only one person showed 2 *SMN2* copies. Also we found the family with *SMN1*-deleted son and his mother. Son was II type SMA patient whereas mother was unaffected. It was estimated that son has 2 *SMN2* copies while mother has 5 *SMN2* copies. Our results confirm genotype-phenotype correlation between the *SMN2* copy number and SMA severity. Extremely high *SMN2* gene copy number may promote the development of asymptomatic phenotype. Thus we conclude that *SMN2* gene copy quantification could be reasonable for precise SMA molecular genetic testing.

P12.204 SMA carrier testing by real-time PCR quantitative analysis of SMN1

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Background: Proximal spinal muscular atrophy (SMA) is one of the most common autosomal recessive diseases, where it has an estimated incidence of 1 in 6000-10000 live births and a carrier frequency of 1 in 40-60. SMA patients are classified into three clinical types based on age of onset and severity of symptoms. Mutations in the survival motor neuron gene 1 (*SMN1*) are determinant for development of the disease, whereas the number of copies of *SMN2* gene plays a role as a phenotypic modifier factor. Approximately 94% of patients have homozygous absence of the *SMN1* whereas most carriers have only one *SMN1* gene copy, this study aimed to establish SMA carrier detection test through *SMN1* quantitative analyses using real-time PCR technique.

Patients and methods: The study included twenty obligate heterozygotes, ten patients with homozygous deletion of *SMN1* and twenty normal controls. Genomic DNA was extracted from peripheral blood samples. Real-time PCR test for *SMN1* gene was optimized and related to that of albumin as a reference gene. The copy number of *SMN1* gene was determined by comparative threshold cycle (Ct) method.

Results: The homozygous *SMN1* deletion ratio of patients was 0.00, the hemizygous *SMN1* deletion ratio of carriers ranged from 0.39-0.59 and about 0.84-2.19 in normal controls.

Conclusion: The study provided accurate and reliable test for SMA carrier detection, genetic counseling and prenatal diagnosis.

P12.205 Genetics of spinocerebellar ataxias in Portuguese patients: screening for SCA15, SCA28, and FXTAS

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The autosomal dominant spinocerebellar ataxias (SCAs) are a heterogeneous group of rare, late-onset neurological disorders caused by progressive neuronal degeneration, mainly affecting the cerebellum. More than 25 SCA loci have been mapped and 18 disease-causing genes identified to date. Mutation screening has allowed us to genetically characterize almost 200 Portuguese ataxia families. However, more than 100 families and an even larger number of apparently sporadic cases, remain without a genetic diagnosis. Here, we present the results of mutation screenings for two recently described SCA subtypes, SCA15 and SCA28, in Portuguese patients clinically diagnosed with dominant ataxia, and the results of FMR1 premutation analysis in males with sporadic late-onset movement disorders. All SCA15 patients identified so far have deletions of at least several exons of the gene ITPR1. SCA28 is caused by point mutations in the gene AFG3L2. The fragile X-associated tremor/ataxia syndrome (FXTAS) is caused by intermediate expansions of CGG repeats in the FMR1 gene.

We carried out quantitative real-time PCR or direct sequencing of the

exons of interest to screen for ITPR1 genomic deletions or SCA28 mutations, respectively, and failed to find any pathogenic alterations. We detected one patient carrying a FMR1 premutation and presenting with a clinical and radiological phenotype compatible with a FXTAS diagnosis.

In conclusion, SCA15 and SCA28 mutations are very rare in the Portuguese population of ataxia patients, while the frequency of FXTAS is very low. A substantial number of SCA genotypes likely remain to be found in the Portuguese ataxia patient population.

P12.206 The analysis of (CAG)_n and (CAT)_n repeats of the ATXN1 gene in spinocerebellar ataxia type 1 patients from Bashkortostan Republic and populations of the Volga-Ural region of Russia.

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Spinocerebellar ataxia type 1 (SCA1) is known to be the most common form of autosomal dominant spinocerebellar ataxias (AD SCAs) in European populations, caused by expansion of (CAG)_n repeats in the coding region of ATXN1 gene. Normal variation of gene contains 6-37 CAG repeats, broken by insertion of 1-3 triplets, which serve as stabilizing factor for (CAG)_n tract. The prevalence of SCA1 in Bashkortostan Republic is 0,07 per 100000 population. The analysis of ATXN1 gene revealed no (CAG)_n alleles without CAT insertions in healthy individuals from Bashkortostan. The control DNA sample with known nucleotide sequence had 4 CAT insertions. Thus, in populations of the Volga-Ural region of Russia there is a combination of low SCA1 frequency and absence (or extreme low frequency) of (CAG)_n alleles of the ATXN1 gene without CAT insertions. Normal ATXN1 gene variation of (CAG)_n repeats is investigated in 12 populations of the Volga-Ural region of Russia: significant heterogeneity of the studied populations is found. We found 13 allele variants with the number of triplets from 22 to 35, the most frequent of them are from 28 to 31, which correspond to number of triplets in the most of European populations. Thus, (CAG)_n repeats of the ATXN1 gene will contribute to diagnostic possibilities as informative genetic marker of populations.

P12.207 mRNA analysis revealed molecular defect of CFTR gene in uncharacterized CF patients

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At present time, CFTR mRNA analysis represents more a research tool for the identification of unknown molecular defects of the CFTR gene in specific rare cases than a routine approach to complete a diagnostic procedure. In particular mRNA analysis may allow researchers to find sequence variations not yet defined like specific splicing defects. Molecular analysis of CFTR performed at DNA level on 800 patients was not able to identify the defect in 101 (6%) CF alleles. In order to characterize these unidentified alleles, 36 cases out of 50 patients were re-contacted and a nasal epithelium sample was collected to analyze the mRNA.

The RNA defects were characterized in about the 30.5 % of analyzed patients. The cDNA analysis showed that 8 patients had a novel transcription product: 4 patients carry an insertion of intron sequence of about 100bp near exon 6b, 2 patients carry a 104bp insertion located between exons 10 and 11; 2 patients presented at DNA level a nucleotide variation described as polymorphism, which instead determines an aberrant splicing at RNA level.

In addition in 2 patients we observed a low level of mRNA product that will be analyzed by quantitative technique. The remaining patients are still under evaluation.

Our results confirm the efficacy of the CFTR analysis at mRNA level as a diagnostic method in characterizing mutations and in checking their effect on normal splicing processes and transcription rates.

P12.208 Sudden unexpected death and genetic analysis of LQTs genes

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Background: The sudden unexpected death in young age is relatively rare and in some cases (4,3% to 50%) it remains unexplained even after autopsy (including toxicology and histology examinations). In these cases, we suppose a malignant arrhythmia to be the leading cause of death. Recent studies showed that genetic disorders of ion channels are responsible for 22% to 28% of sudden deaths. Most commonly, the Long QT syndrome (LQTS) is present, but also some other disorders, like catecholaminergic polymorphic ventricular tachycardia (CPVT) and hypertrophic cardiomyopathy (HCM) have been described.

The aim of the project is to apply genetic analysis (in these selected group we intended to perform mutation analysis of the following genes: KCNQ1, KCNH2, KCNE1, SCN5A, KCNE2 and ANK2 genes) in cases of sudden unexpected deaths under 40 years of age, to discover the occurrence of particular genetic disturbances related to malignant arrhythmias (ion channels diseases - LQTS and CPVT, HCM), and to perform clinical examination of surviving relatives aimed to identify the families endangered by sudden cardiac death. We suppose, that from all cases of sudden death without pathological findings, we will discover at least 20% to be caused by ion channel genetic disorder (LQTS, CPVT)

Conclusion: Combining the genetic examination of dead subjects with examination of first-degree relatives (ECG, echocardiography, exercise ECG testing, clinical examination) is possible to identify the cause of 40% of sudden unexpected deaths.

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P12.209 Molecular analysis of COCH gene in patients with superior semicircular canal dehiscence

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Superior semicircular canal dehiscence (SSCD) was originally described by Minor and colleagues in 1998, and it is characterised by the absence of bone overlying the superior semicircular canal, which creates a third labyrinthine window (with the oval and round windows). The consequence is the loss of acoustic energy and abnormal stimulation of the vestibular system; the clinical manifestations include Val-salva- and exercise-induced vertigo, sound-induced vertigo (tullio phenomenon), and variable conductive hearing loss. It is important to detect SSCD because it could be partially or fully corrected with surgery. The genetics of SSCD have not been studied. On the contrary, autosomal dominant nonsyndromic hearing loss at the DFNA9 locus (characterised by vestibular dysfunction) is known to be caused by mutations in the COCH gene (chromosome 14q12), which encodes cochlin. Recently, an association between a mutation in the COCH gene and the presence of SSCD has been reported (Am J Med Genet 2009; 149A: 280-5).

We performed complete sequencing of COCH gene in 3 patients with proven SSCD, previously diagnosed by CT and surgically corrected. We didn't find any nucleotide change in the COCH gene sequence. The molecular basis of this rare disorder remains to be elucidated.

P12.210 Frequency of positive *Xmn1* Gy polymorphism and coinheritance of common alpha thalassemia mutations do not show statistically significant difference between thalassemia major and intermedia cases with homozygous IVSII-1 mutation

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From 362 thalassemia cases referred to Adult thalassemia clinic of the Iranian blood transfusion organization (IBTO) for genotyping and further studies, 103 cases (28.5%) had a common primary disease factor, IVSII-1 mutation in homozygous state. 61 (59.2%) of these individuals represented thalassemia major and 42 (40.8%) thalassemia interme-

dia clinical phenotype. To re-evaluate our current diagnostic criteria, *Xmn1* γ polymorphism and coexistence of alpha thalassemia mutations, frequently recalled as important factors modifying the clinical phenotype of homozygous beta zero thalassemia cases in our country, were examined in both groups. No statistically significant difference in the frequency of positive *Xmn1* γ polymorphism was observed between thalassemia intermedia and thalassemia major patients. Double gene deletion α -Med was observed in only one thalassemia major case, while α -aa^{3.7} in heterozygous state (α -aa^{3.7}) was identified in 6 (9.8%) of thalassemia major and 8 (19%) of thalassemia intermedia patients and α -aa^{4.2} was observed in only one thalassemia major case. No statistically significant difference in the frequency of coinheritance of alpha thalassemia was observed between the two groups. These results imply that other interacting mechanisms which modify the phenotype of thalassemia patients is still in the dark in our current diagnostic criteria of thalassemia.

P12.211 Development of the oral cavity : from gene to clinical expression in human

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Pathologies or developmental anomalies of the oral cavity are one clinical aspect, leading eventually to diagnosis, but often underestimated and considered, especially in their management, of genetic diseases or syndromes. Among more than 7000 known rare diseases, 850 have dental/oral/facial manifestations and more than 300 include in their clinical synopsis a cleft lip/palate.

This original research project offers to combine complementary and convergent approaches in bioinformatics, developmental biology especially through the study of genetically engineered mice, to improve the knowledge and understanding of the formation of the oral cavity and specifically of the palate and dentition. It uses the following approaches:

- 1) Selection of known genes responsible for rare diseases but for which expression and/or roles are insufficiently characterised.
- (2) Identification of new candidate genes, through a systematic analysis of their craniofacial and dental expression patterns using the EURExpress mouse transcriptome-wide atlas (<http://www.eurexpress.org/ee/>) and the "Odontogenesis" related database.
- (3) A detailed analysis of the expression patterns of the most interesting genes, is performed by *in situ* hybridisation techniques, in the mouse at various stages of odontogenesis.
- (4) Animal models craniofacial and orodental phenotypes are detailed using an imaging platform (micro-CT)
- (5) *In vitro* organ culture and manipulation

This work federates scientists and clinicians and should stimulate the implementation of science based evidence diagnosis and new therapeutic options (treatment of teeth agenesis by stimulation of odontogenesis *in situ*; tissue engineering) thus contributing to the wellbeing and orodental and general health care of the patient (Grants APIHUS, IFRO, UdS, MAEE PHC Thai).

P12.212 Identification of gross deletions in *TCOF1*: Use of MLPA in the diagnosis of Treacher Collins-Franceschetti syndrome.

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Treacher Collins-Franceschetti syndrome (TCS) is an autosomal dominant craniofacial disorder characterised by midface hypoplasia, micrognathia, downslanting palpebral fissures, eyelid coloboma, and ear deformities that often lead to conductive deafness. High inter- and intra-familial clinical variability is observed, ranging from perinatal death due to a compromised airway to a phenotype that goes undetected by medical examination. TCS is caused by null mutations in the *TCOF1* gene.

The Oxford Molecular Genetics Laboratory provides a specialist molecular genetics service for rare craniofacial and skeletal disorders, which includes TCS. Our testing strategy is to sequence the 27 coding

exons of *TCOF1* which will detect point mutations, splicing mutations and small indels. Patients with classical TCS without a *TCOF1* sequence variant may reflect genetic heterogeneity; however, no alternative genes have been identified. Alternatively, it may indicate that, due to incomplete gene screening, certain *TCOF1* variants remain undetected. We therefore hypothesised that gene rearrangements may underlie a proportion of TCS cases without a molecular diagnosis. We collaborated with MRC-Holland to develop an MLPA kit that tests *TCOF1* for gene rearrangements. After test validation, patients referred for *TCOF1* analysis but tested negative by sequencing were tested. Out of 53 patients tested, 5 (9.4%) were found to have a deletion involving part of *TCOF1*. This is the first report of gross deletions resulting in TCS, and indicates that gene rearrangements do account for a significant proportion of cases.

P12.213 A novel nonsense mutation in *UNC13D* causing a severe form of Familial Hemophagocytic Lymphohistiocytosis.

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Familial hemophagocytic lymphohistiocytosis (FHLH) is a life-threatening autosomal recessive disorder of immune regulation. It is characterized by severe hyperinflammation caused by the uncontrolled proliferation of activated lymphocytes and histiocytes. HLH is a heterogeneous disease with regard to genotype and phenotype. Four genes *PRF1*, *UNC13D*, *STX11*, and *STXBP2* have been linked to the disease so far. Here we report the characterization of a novel nonsense mutation in the *UNC13D* gene that was detected in three unrelated patients.

All three patients were admitted to the hospital at very young age (6, 8 and 12 weeks old) and fulfilled the criteria of HLH, i.e. fever, cytopenia, (hepatosplenomegaly, hemophagocytosis in central nervous system (CNS), elevated levels of ferritin and soluble CD25. Treatment was given according to the HLH 2004 protocol, but all three infants deceased at an age of 5 to 6 months. Homozygosity for the c. 2695C>T (p.Arg899X) mutation in exon 28 of *UNC13D* was shown in two patients. The third patient showed compound heterozygosity for the c.2695C>T (p.Arg899X) and another mutation in *UNC13D*. In patient tissues *UNC13D* mRNA was present. Further investigation revealed that the mutation resulted in a misfolded dysfunctional protein. Geographical clustering of the patient families and genealogical research, where two patients could be traced to a common link, suggests that a single ancestral founder might have introduced the mutation in the Netherlands. Conclusion: the novel c.2695C>T (p.Arg899X) mutation in *UNC13D*, which most likely originates from a common founder is associated with severe early lethal HLH.

P12.214 Genetics and Clinical Aspects of Usher and Pendred Syndromes in Iranian Population

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Syndromic hearing impairment accounts for 30% of prelingual deafness. The two most common types are Usher and Pendred syndromes both of them have AR pattern of inheritance. Three different types of Usher syndrome USH I (50%), USHII (35%) and USH III (15%) have been recognized. Up to now 13 different loci and 8 genes have been identified. Also Pendred is characterized by congenital sensorineural hearing loss, goiter (40-60%) and inner ear abnormalities and impaired vestibular function test. The objective of this study was to identify the prevalence of USH loci in deaf-blind patients and PDS gene mutations

in Iranian Population.

Thirty USH families and 120 families with Pendred syndrome were subjected to linkage analysis using STR markers. The sequencing for mutation screening was performed for the linked families. eleven out of thirty USH families were linked to the studied USH loci which we identified the mutation in four of these families and mutation screening in the other linked families is on the way. Also we were able to link fourteen autosomal recessive hearing impaired families to DFNB 4 locus and nine of them showed different types of PDS mutations. In conclusion 33% of the families with USH syndrome were linked to one of the known loci, however additional study needed to determine the causative genes involve in the other families. We have also been able to determine Pendred syndrome as the second cause of hearing loss in our population.

P12.215 Molecular characterization of Iranian patients with type 3 von Willebrand disease

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von Willebrand's disease (VWD) type 3 is a rare but severe autosomal-recessive inherited bleeding disorder with a prevalence higher in certain locations where consanguineous marriages are relatively frequent. The genetic defects causing recessive type 3 VWD in 10 unrelated families from Iran have been investigated and the genetic heterogeneity among these patients was evaluated. All exons and their flanking regions of von Willebrand factor gene were amplified by PCR and sequenced using specific primers. Eight patients were fully characterized at the molecular level. Six different gene alterations were identified. All the mutations caused null alleles, three being non-sense mutations (Q104X, Q793X and E1981X), two possible splice site mutations (2443-1G>C and 1110-1G>A) and one small deletion (3237delA). Three of them have not been described previously. Most patients were born from consanguineous marriages and all were homozygous for their mutations. The results confirm that molecular defects in type 3 VWD are heterogeneous with mutations arising randomly within the entire gene.

P12.216 Dissecting the genetic heterogeneity in Walker-Warburg syndrome and related cobblestone lissencephalies

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Walker Warburg syndrome (WWS; OMIM 236670) is an autosomal recessive disorder characterized by muscular dystrophy, eye and neural migration defects and other structural brain anomalies, such as cerebellar hypoplasia and hydrocephalus. Other WWS-like disorders include Fukuyama Congenital Muscular Dystrophy (FCMD, OMIM 253800), and Muscle-Eye-Brain disease (MEB, OMIM 253280). A common feature of these syndromes is neural overmigration during neocortex lamination, giving rise to cobblestone lissencephaly, disorganized cerebral cortices and multiple coarse gyri with agryic regions. Mutations in six genes have been associated with cobblestone lissencephaly. Mutations in these genes commonly result in decreased O-linked glycosylation of alpha-dystroglycan. The six known genes account for approximately 1/3 of cases of WWS, suggesting that there are significant number of genes yet to be identified. At least a percentage of these are hypothesized to be involved in O-linked glycosylation.

Genetic and functional genomics approaches are being applied to identify the remaining WWS genes. Homozygous regions in 30 consanguineous and outbred families with one or more affected individuals have been identified by a homozygosity mapping approach. Our data are consistent with at least ten additional WWS genes being present. Additional novel methodologies for gene identification include next generation sequencing and comparative expression profiling in fibroblasts from patients and unaffected siblings are in progress. We anticipate that the results of the combined studies will identify additional genes that are crucial for glycosylation of dystroglycan, which will have

an important impact on diagnostic testing and genetic counseling and increase our fundamental knowledge on this glycosylation pathway.

P12.217 Haplotype mapping of the Welander distal myopathy region on chromosome 2p13.

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Welander distal myopathy (WDM) (MIM 604454) is an autosomal dominant inherited muscle dystrophy with late onset. The disorder is characterized by progressive weakening of the distal limbs with extensor muscles in hands and feet first afflicted. Disease onset is usually around 40-60 years of age and the incidence is high in regions of Sweden and Finland. A candidate region on chromosome 2p13 flanked by marker D2S358 and D2S2835 has previously been reported (von Tell et al. 2003) but no disease causing mutation has been identified. Novel genome assemblies show that the candidate gene region is larger and contains more genes than first anticipated.

We investigated 26 non-related and affected individuals originating from Sweden and Finland. Affected individuals were genotyped using 13 microsatellites spanning the candidate region on chromosome 2p13 confirming an association to WDM. All patients share a common haplotype between marker D2S358 and D2S291 spanning 2,1cM. The size of the shared region is 3,0 Mb containing 55 annotated genes. The conserved haplotype segregating disease suggests a founder effect with an estimated age of 95 generations (calculated as 2/g morgans) (Boehnke 1994). We hypothesize that a large chromosomal rearrangement, e.g. an inversion, may explain the conserved haplotype. Such a rearrangement may also disrupt a gene at one of the inversion breakpoints. The haplotype segregating WDM is now being restricted in search for candidate genes.

P12.218 Strategy for mutation detection in Serbian patients with Wilson disease

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Wilson disease (WD) is an autosomal recessive disorder of copper metabolism resulting in pathological accumulation of copper in many organs and tissues. This condition is caused by mutations in the gene coding for a copper-transporting CPx-type ATPase (ATP7B). More than 300 distinct mutations of ATP7B are described, but in most populations majority of the WD cases are due to a small number of specific gene changes. In Central and Eastern Europe the most common mutations are located in exons 14, 8, 5, 13 and 15. That has already been confirmed for Serbian WD patients in pilot study realized in Italy. Therefore, as the first step in establishing molecular genetic testing for WD in our country, we performed direct sequencing of those five exons of ATP7B gene. We analyzed DNA samples of 34 unrelated Serbian WD patients using ABI 310 Genetic Analyzer. Total number of 43 mutated alleles were found (63.2%). 17 patients (50%) carried two mutations in ATP7B gene (homozygous or compound heterozygous), while 9 patients (26.5%) carried only one ATP7B mutation. In 8 patients (23.5%) no mutations were found. We detected 11 different mutations, one of which is novel. The most common mutations are: H1069Q (39.5%), 2304_2305insC (25.6%), A1003T (11.6%), and R616Q (7%).

These results indicate that sequencing of exons 14, 8, 5, 13 and 15 is good start strategy for mutation detection in WD patients in Serbia. However, mutation analysis of the remaining exons in ATP7B gene should be developed.

P12.219 Mutational analysis and genotype-phenotype correlation of Wilson disease patients in an isolated Romanian population

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Wilson disease is a rare condition characterized by a defect in excretion of copper, due to a mutation of both alleles of ATP7B gene. The mutations in the ATP7B gene lead to intracellular accumulation of copper and severe hepatic and neurological abnormalities. The human ATOX1 protein is an metallochaperone that interacts directly with copper-transporting ATP-ase (ATP7B) and can regulate its copper occupancy. We report the further results of an ongoing project

concerning the spectrum of mutations of ATP7B and ATOX1 genes in WD patients and their relatives (5 WD patients and 152 autochthonous inhabitants to the third generation originated from the same village) from an isolated Romanian populations with high prevalence of WD. Patients' demographic, clinical and histopathological parameters were obtained. Direct sequencing of all 21 exons of ATP7B shown that four WD patients are heterozygotes or compound heterozygotes for three mutations P767P-fs, H1069Q or K832R and one WD patient was homozygous for K832R. Mutation analysis of the four exons of the ATOX1 gene including the intron- exon boundaries revealed one known polymorphism (5'UTR -99 T>C) in 27 out of 157 autochthonous inhabitants. Based on the data of this study, no major role can be attributed to ATOX1 in the pathophysiology or clinical variation of Wilson disease. Phenotype variation among subjects with the same ATP7B genotype suggests that modifying factors play an additional role in the pathogenesis of WD.

P12.220 Novel missense mutation in WISP3 gene associated with childhood onset progressive pseudorheumatoid arthropathy (c.667 T>G)

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Childhood onset progressive pseudorheumatoid arthropathy (OMIM#208230) was first described by Spranger et al. (1983) as an arthropathy of childhood beginning at about age 3 to 8 years with progressive joint stiffness. This condition is characterized by that it first affects the hips with pseudorheumatoid manifestations i.e. morning stiffness and decreased mobility of the cervical spine. Hurvitz et al. (1999) described mutations in WISP3 gene family members as causative for the disease.

Here we report a novel familial mutation within WISP 3 gene most probably associated with the condition clinically recognized as spondyloepiphyseal dysplasia tarda with progressive arthropathy and sent for familial genetic testing.

Mutation was detected by gene screening approach using direct automatic sequencing. This information was utilized for development of PCR based assay for familial genotyping. Mutation in homozygous form in index-patient and as heterozygous in patients was confirmed implying the actual etiopathological basis for progressive pseudorheumatoid arthropathy in this family. For definite confirmation of this hypothesis, functional analysis should be performed.

P12.221 A novel WT1 mutation identified in a patient with steroid-resistant nephrotic syndrome

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Idiopathic nephritic syndrome is the most frequent disease of the glomerular ultra-filter in children. The established treatment of the affected individuals includes corticosteroid administration, which, unfortunately, fails to give positive result in app. 10% of the cases and, as a result, the children often progress towards end-stage renal disease. Several genes have been shown to be involved in the pathogenesis of this steroid-resistant form of nephrotic syndrome (SRNS). Among those, most commonly affected are NPHS1, NPHS2 and WT1. Here, we report the identification of a novel WT1 mutation in a girl diagnosed with SRNS at the age of 2. The initial genetic screening revealed no mutation in the NPHS2 gene, which prompted us to look for defects in the WT1 gene. A heterozygous nucleotide change, C1184T in exon 9, resulting in amino acid substitution Ser395Tyr was identified. In order to determine whether this novel variant is indeed a disease causing change we checked if it was present in healthy controls. The substitution was not found in any of the 240 chromosomes tested. The affected amino acid, Ser395, was found to be conserved among multiple species as diverse as Drosophila, Xenopus and human. It is located in zink-finger 3, part of the DNA binding domain of WT1. The absence of the variant among healthy population controls and the fact that it involves residue located in a conserved part of the protein, allowed us to conclude that it is, indeed, the disease causing mutation in this case.

P12.222 The Tunisian experience in X linked mental retardation

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Mental retardation is the most frequent cause of handicap. Linkage studies followed by mutational analysis of known MRX genes localized within defined genetic intervals represent a good strategy to identify a genetic cause of the disorder. We report the Tunisian experience in X linked mental retardation. Our study of more than 10 families has concluded on three important families. The first one was the MRX54 including 14 males which allowed us to identify a new mutation on the ARX gene a substitution of a leucine (CTG) with proline (CCG) on the amino acid number 33(L33P) .The second family shows moderate mental retardation with behavioral symptoms, common dysmorphic features. Linkage analysis showed that affected males and obligate carrier females share a common haplotype in the Xp21.31 - Xq23 region that contains PAK3 gene. Sequencing of PAK3 allowed us to identify the first splice mutation in PAK3 gene located at the 5' end of intron 6 (c.276+4A>G). The third family including 3 males with severe to mild mental retardation, short stature, lean body and microcephaly. The disease was mapped into an interval encompassing Xp21.1-Xq21.33 (maximum LOD score of 0.90). Mutation analysis of genes located here allowed us to identify a truncating mutation in the PQBP1 gene. This mutation is an insertion of a one adenosine residue in exon 5 (c.631insA). This frameshift insertion causes premature stop codon at amino acid position 226. This study shows that small families too must be explored despite their non informativity and mutations can be identified.

P12.223 Molecular investigation of a novel clinical expression of Xeroderma Pigmentosum in a Tunisian family

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Xeroderma Pigmentosum is a rare genodermatosis predisposing to skin cancers. The disease is classified into eight groups. Among them, the *Xeroderma Pigmentosum* group A (XP-A), which is characterized by the presence of neurological abnormalities in addition to cutaneous symptoms.

In the present study, we report on a particular XPA family where some members showed an atypical clinical presentation i.e. unlabelled neurological abnormalities with discrete skin manifestations. Dermatological examination showed a small number of monomorphic and discrete pigmented maculae, which were not evocative of the clinical diagnosis of XP.

Molecular investigation allowed identification of a novel XPA mutation p.V241GfsX5. All patients with this novel phenotypic expression of XPA are compound heterozygous for (p.[R228X(+).V241GfsX5]) mutations.

Our report shows that mutations in the XPA gene could lead to other phenotypic expressions. We suggest that this phenotype should be considered as a novel clinical entity allelic to XP-A.

J12.1 Molecular analysis of phenylketonuria in Belarus

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Phenylketonuria (PKU) is an autosomal recessive disease caused by mutations in phenylalanine hydroxylase (PAH) gene (MIM#261600). PKU is the most common inborn error of amino acid metabolism in Belarus (1/6000). Nowadays more than 500 mutations are known in PAH locus. The major mutation in our country is R408W, which is routinely screened in PKU patients since 1992, having frequency 68%.

In order to explore the spectrum of mutations, we conducted the study of PKU chromosomes with previously unknown primary gene defect. At first step we performed RFLP analysis for the most common Eu-

ropean mutations. The established frequencies were: R158Q - 6,6% (37/564), R261Q - 1,6% (9/564), IVS12nt1 - 1,2% (7/564), IVS10nt11 - 0,4 % (2/564) and Y414C - 0,5% (3/564).

Sequencing of full coding region of PAH gene in patients with unidentified mutations is now in progress. Our research revealed that mutations of the exon 7 cover about 6% of alleles in Belarusian PKU patients. We found 6 different nucleotide substitutions in 21 patient with following frequencies: E280K -1,7% (10/564), R252W - 1,2% (7/564), P281L - 0,5% (3/564), R243X - 0,4 % (2/564), R241C and G272X - 0,2% (1/564).

Identified mutations represent 82% of PKU alleles. Currently, prenatal diagnosis can be done in 97% of high-risk families by direct mutation analysis of the PAH gene and indirect analysis of STR and VNTR polymorphic sequences.

P13 Metabolic disorders

P13.01 Segregation of four new STRs in 28 Tunisian patients affected by 11 β Hydroxylase Deficiency

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Eleven beta hydroxylase deficiency (11βHD), is the second most common form of congenital adrenal hyperplasia (CAH), accounting for 5-8%. It's an autosomal recessive enzyme defect impairing the biosynthesis of cortisol. In Tunisia, the incidence of 11 β hydroxylase deficiency appears higher (17,5% of congenital adrenal hyperplasia etiology). The identical presentation of genital ambiguity (females) and pseudo-precocious puberty (males) can lead to misdiagnosis with 21 hydroxylase deficiency. The clinical hallmark of 11β hydroxylase deficiency is variable, and biochemical identification of elevated precursor metabolites is not readily available. To avoid these problems, genetic analysis of CYP11B1 gene is an alternative diagnostic test.

In the present study, we performed a molecular genetic analysis of CYP11B1 gene in 28 Tunisian patients clinically affected by 11β hydroxylase deficiency. They belong to 26 families originate from the Center of the country. We studied four extra-genic microsatellites markers; MI8S501, MI8S502, MI8S302 and MI8S301, surrounding the CYP11B1 gene (localized on 8q21-22).

Our results showed 4 different haplotypes shared by all the families. They seem to come from one common ancestor. In addition, exons sequencing of CYP11B1 gene reveals a rare and presumably specific "G379V" mutation probably specific for Tunisian population.

P13.02 The prevalence of alpha-1-antitrypsin deficiency in a representative population sample from Slovakia

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Alpha-1-antitrypsin (A1AT) deficiency is reported as one of the most frequent autosomal recessive genetic disorders in Caucasians. Aberrant A1AT protein leads to emphysema and chronic obstructive pulmonary disease or prolonged neonatal jaundice, cirrhosis and cancer. Hence the primary aim of the present study was to screen entire coding sequence of A1AT gene for causative mutations in 24 affected patients in Slovakia. Three different deficient alleles were identified in the study: Mprocida (c.T693C, p.L65P) and S (c.A1362T, p.E288V) in single copy; and Z allele (c.G1595A, p.E366K) in 9 copies (only one homozygous state, estimated frequency 0.02). In order to assess an unbiased frequency of Z allele in Slovak population, we have designed rapid and cost effective BI-PASA PCR test method. In the population sample of 338 adult subjects 10 Z allele carrying heterozygotes have been identified, giving allele frequency to be of 0.0148. Therefore, the estimated prevalence of Z homozygotes in Slovakia is 1/4600; and thus expected number of subjects with severe A1AT deficiency in the whole population of Slovakia (5.5 millions) is about 1200. Considering less frequent pathogenic alleles (not tested in population in this study)

and their combinations with Z allele the real frequency may be even higher. These findings underlie the need for genetic testing being introduced into a common clinical practice.

P13.03 A Mitochondrial DNA Mutation (T4454C) in the tRNA^{Met} Gene in Iranian Patients with Brugada Syndrome

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Point mutations in mitochondrial tRNAs can cause severe multisystemic disorders and impaired mitochondrial function. Recently interesting genetic data became available regarding the importance of mtDNA mutations in the etiology of arrhythmia and cardiac function. Brugada syndrome is clinically cause sudden death in young ages. We report here mutational analysis of mtDNA from 15 unrelated patients with Brugada syndrome and identified a homoplasmic tRNA^{Met} 4454 T>C mutation in 8 cases with variable severity and 18- 41 years old. All of them have typical Brugada- type ECG changes. This sequence change is located in the T-stem loop of tRNA, a moderately conserved region. It was not found in 45 local controls and previously in other disorders reported as a polymorphism. It was considered non- pathologic but we suggest that the high level conservation in secondary and tertiary structures of this tRNA, make the tRNA^{Met} as unique case because only one type of this tRNA encoded in the mammalian mtDNAs. Thus any changes in primary sequence may potentially pathologic in cardiac cells but further investigations necessary to clarify this correlation.

P13.04 Analysis of CYP21A2 gene mutations in Iranian population

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Congenital adrenal hyperplasia (CAH) is comprised of a family of autosomal recessive disorders leading to the defect in cortisol biosynthesis. The 21-hydroxylase deficiency is the most common cause of CAH which accounts for 90% of the CAH patients. There are various phenotypes due to combination of different mutations. Eight common mutations in CYP21A2 gene, located on 6q21.3, have been reported in many populations around the world. We have studied the spectrum of CYP21A2 mutations including the most common mutations, rare mutations, large deletions, duplications and gene conversions in Iranian patients affected by CAH. 30 CAH affected families were included in the study. Allele-specific amplification of CYP21A2 gene was performed to detect the common mutations. Dosage analysis of the affected individuals was done to find out deletions and duplications by multiplex ligation probe amplification (MLPA) method. Also, sequencing procedure was applied for the remaining mutations. The allele specific amplification assay detected p.P30L, g.655A>C>G (I2G), p.G110_Y112, p.I172N, Cluster 6 (p.I236N, p.V237G, p.M239L), p.V281L, p.Q318X, and p.R356W. Some affected individuals carried partial gene conversion, duplication and others were carrier of deletion resulting in a chimeric CYP21A1P-CYP21A2 gene. Due to high prevalence of consanguineous marriage in Iran, the recurrence risk of the disease is high. Screening the heterozygotes of at risk families would help to diminish the psychological and economical stress these families would deal with through out their life. Moreover, these findings could be used for prenatal diagnosis and detecting the heterozygotes in non-related CAH families without having a previously CAH affected child.

P13.05 Congenital adrenal hyperplasia in Alexandria, Egypt: A high prevalence justifying the need for a community-based newborn screening program

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Gynaecology Department, Alexandria faculty of Medicine, Alexandria, Egypt. Congenital adrenal hyperplasia (CAH) is one of the most common inborn endocrine disorders. Its prevalence ranges between 1:23,344 (New Zealand) and 1:282 (Alaska). CAH is world-wide increasingly being included in newborn screening programs. Benefits of screening are avoidance of salt loss crises and early proper gender assignment in virilized girls. To determine the prevalence of CAH in Alexandria, Egypt, a total of 7254 samples from newborns at 5-7 days of age were screened by time resolved fluoroimmunoassay (DELFIA; PerkinElmer) for 17OHP determination from June, 2008 to May, 2009 at SMC, Alexandria, Egypt. Neonates with a value > 30 nmol/l were considered positive. Six cases were diagnosed as positive. Only one recall was made giving a very low recall rate (0.00014). Prevalence of CAH in Alexandria, Egypt was 0.08 % (1/1209) with a sensitivity of 100 %, specificity of 99.99 %, and a positive predictive value of 0.86. One affected female newborn had the simple virilizing form, 2 were salt wasting, and one had the non-classic (NC) form of the disease. One male died in the neonatal period from an adrenal crisis, and another male had the NC type of the disease. All the positive cases were reported to the ministry of health to start immediate treatment for salt loss and genital ambiguity. Implementation of a community-based newborn screening program for CAH in Egypt is justified due to the efficiency of time resolved fluoroimmunoassay for screening in the neonates, and the high frequency of CAH and its serious complications if untreated.

P13.06 GROWTH FACTOR SIGNALING PROMOTES TUMOR METABOLISM BY REGULATING EXPRESSION AND ACTIVITY OF KEY ENZYMES

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Growth factor signaling promotes cellular proliferation. It does so by promoting anabolic metabolism in proliferating cells and hence it positively drives the metabolic needs of tumor cells. We have investigated how growth factor signaling promotes anabolic tumor metabolism by studying the effect of its inhibition on expression and activity of key enzymes- pyruvate kinase M2 (PKM2) and transketolase like enzyme-1 (TKTL1), known to be important for tumor metabolism. On inhibiting growth factor signaling, we have observed decreased mRNA levels of both enzymes but increased activity of PKM2 compared to control in treated cell lines as judged by quantitative PCR and spectrophotometric assay respectively. Expression of high activity PKM2 form and down regulation of TKTL1 expression inhibits tumor metabolism as judged by decreased cellular proliferation. This correlates with previously reported observation that expression of low activity PKM2 form and up-regulation of TKTL1 is an important event in metabolic transformation. Our results demonstrate that inhibition of growth factor signaling has a negative effect on tumor metabolism by modulating expression of TKTL1 and expression/activity of PKM2.

P13.07 MTHFR C677T Polymorphism in Russian Patients with Cystic Fibrosis

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MTHFR C677T polymorphism has been recognized as one of key regulatory factors of both homocysteine level and oxidative stress response. Objectives: (1) study on MTHFR C677T alleles distribution in patients with cystic fibrosis (CF) and (2) evaluation of correlation of MTHFR C677T polymorphism with clinical presentation (severity of the disease and exacerbations frequency during last six months) along with biochemical parameters (blood levels of glutathione, glutathione peroxidase, zinc, uric acid, and homocysteine). Material and methods: CF patients group comprised 13 females (average age 11.0 yo) and 16 males (average age 12.6 yo). Severity of the disease was estimated as a frequency of pulmonary exacerbations during last six months. All patients were tested for a presence of 21 mutations of CFTR (delf508, dell507, del21kb, 394delTT, R334X, R347P, G542X, G551d, R553X, N1303K, 2143delT, 2184insA, 2113delA, 2118del4, 2141insA, delE672, 2176insC, 2183AA/G, 2183delAA, 2184delA, W128R). MTHFR C677T

polymorphism was determined by PCR-RFLP analysis. Results: Eighteen of 29 (62%) CF patients were homozygous for C-allele of MTHFR C677T, 8 (28%) had heterozygous CT genotype, and 3 (10%) were carriers of homozygotes for T-allele. Correlation analysis did not reveal any parameter, including homocysteine level, to be associated with MTHFR C677T status in CF patients. However, a difference in pulmonary exacerbations frequency between carriers of delf508 mutation (n=23) and non-carriers (n=6) was noted (average 2.8 and 1.5, respectively). Conclusion: This study failed to demonstrate an appreciable association of clinical presentation of CF and biochemical parameters with MTHFR C677T polymorphism.

P13.08 Clinical and molecular analysis of a large Italian cohort of CHI patients

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⁶Pediatrics-Department of Reproductive and Development Sciences, Trieste, Italy. Congenital hyperinsulinism (CHI) is a common cause of hypoglycaemia in infancy. The prevalence of the disease is approximately 1/50000 live births. Hypoglycaemia can cause systemic manifestations and symptoms in the CNS. Histologically there are primarily two forms: a diffuse form, responsible for 60-70% of cases and a focal form, responsible for the remaining 30-40%. HH is caused by mutation in ABCC8, KCNJ11, GLUD1, GCK and HADH genes. In addition to expressing the disease, patients with GLUD1 mutations have hyperammonaemia and those with HADH mutations showed raised plasma hydroxybutyrylcarnitine and urinary 3-hydroxyglutarate. The CHI is most commonly inherited in an autosomal recessive manner and less commonly in an autosomal dominant manner. No really genotype-phenotype correlations have appeared until now. In our study, we enrolled 34 patients from different Italian Hospitals, 27 patients having classical CHI and 4 having hyperinsulinism / hyperammonaemia (HI/HA). Anamnestic data for all patients were collected. For all patients we analyzed all the genes CHI-related by direct sequencing. We found 16 mutations in 13 patients with classical CHI (Detection Rate 48%) and 5 mutations in 7 HA/HI patients (Detection Rate 71%). Most of the mutations are missense mutations (62%), but also stop codon (14%), altered splicing site (5%), insertion (9,5%) and insertion-deletion (9,5%) were detected. All missense mutations involve aminoacid highly conserved across species and none of the 100 healthy controls analyzed DNAs showed the same variant. Finally, this is the first clinical and molecular analysis of a large Italian cohort of CHI patients.

P13.09 Linking cholesterol metabolism and human behaviour

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Introduction: The molecular bases of human behavior have largely eluded identification using standard diagnostic criteria. This likely arises because standard diagnoses are amalgams of symptoms and signs that are the product of several underlying causal mechanisms. In contrast, dissection of human behavior into unidimensional, homogenous traits should enable identification of coherent, causally active psychological entities.

Methodology: Testing this, we identified an X-linked disorder of cholesterol metabolism with intellectual and behavioral abnormalities that establishes this link. Affected males have CK syndrome, a syndromic intellectual disability, whereas affected females segregate high levels of callousness, a component of psychopathy.

Results: Partial loss-of-function mutations of *NSDHL* (NAD(P)H steroid dehydrogenase-like) are responsible for this disorder.

Conclusion: Thus we establish a genetic link between human behavior and cholesterol metabolism and demonstrate that dissection of human behavior into homogenous traits allows ready identification of genetic factors modulating behavior.

P13.10 Altered expression profiles of human clock genes

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Circadian rhythms in physiology and behavior are observed in all mammals, including humans. These rhythms are generated by circadian clocks located in the hypothalamus and also in most peripheral tissues. Recently, clock genes were identified as the genes that control a vast array of circadian rhythms. In our study we investigated the circadian expression of clock genes (*PER1*, *PER2*, *PER3*, *CLOCK*, *BMAL1*, *CRY1*) in human whole blood cells of 10 patients following surgery and exposed to a 24h artificial light. Clock genes were found to oscillate throughout the light-dark cycle. Severe disturbance of the endocrine rhythm as well as clock gene expression profiles in peripheral blood mononuclear were noticed. This deregulation might be the result of the direct effects of injury on the biological clock and the absence of synchronizer essentially light/dark rhythm.

P13.11 New mutations of CYP21 in Turkish population

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Congenital Adrenal Hyperplasia (CAH) is an autosomal recessive disease and most frequently occurs as a result of deficiency in 21 hydroxylase enzyme. Many mutations of steroid 21 hydroxylase enzyme gene (CYP21) have been reported before. A/C659G, 8bp deletion, complete deletion of exon 3, P30L, I172N, exon 6 cluster (I236N, V237E, M239K), V281L, Q318X and R356W are most frequent mutations. Although incidence of the disease is not known in Turkey, frequency of these nine mutations among CAH patients with classic forms (classic salt-wasting and classic simple virilizing) was found as 75 %. 264 patients diagnosed as CAH according to clinical and laboratory findings were admitted to our department for genetic testing. These known nine mutations were tested by PCR and RLFP in peripheral blood DNA sample of these patients. We did not find any of these mutations in 33 of 47 patients who have classic CAH phenotype. These mutations were found as heterozygous form in 14 of these patients. The alleles without any mutations were sequenced. The sequencing analysis results will be presented.

P13.12 Detection of the polymorphisms in the CYP2D6 gene and their influence on the metabolism of drugs

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The response to many drugs in common use varies greatly among patients. The cytochrome P450 2D6 (CYP2D6) is an enzyme responsible for metabolism of many commonly used drugs such as antidepressants, neuroleptics, beta blockers and antiarrhythmics. The gene *CYP2D6* (22q13.1-13.1) that encodes this enzyme is highly polymorphic and shows a great inter-individual and inter-ethnic variability. The polymorphisms lead to different individual responses which result in an increased risk of adverse drug reactions or the lack of the therapeutic response. According to the enzymatic activity the Caucasian population can be classified into four subgroups - (1) poor metabolizers, who degrade the CYP2D6 substrates slowly and who are exposed to a greater incidence of side effects of therapy, (2) extensive metabolizers, their metabolism follows the presupposed mechanism, (3) intermediate metabolizers, the subgroup between poor and effective metabolizers, and (4) ultrarapid metabolizers, their metabolism is without adequate clinical response to common drug dosages. The influence

of polymorphisms on paroxetine and risperidone treatment is studied at Department of Medical Genetics and Psychiatric clinic. Polymorphisms occurring in coding part of *CYP2D6* gene are determined by sequencing, Real Time PCR, High Resolution Melting analysis and agarose electrophoresis. This paper provides an overview of current technologies available for assessing *CYP2D6* polymorphisms at Department of Medical Genetics.

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P13.13 Risk factors for cystic fibrosis liver disease

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Introduction: Cystic fibrosis(CF) associated liver disease is the second cause of death in CF and may be the first disease expression in CF. It seems that many recognized risk factors like severe mutations history of meconium ileus and male gender could suggest the occurrence of the liver disease.

Aims & Methods: Aim study was to assess liver disease's frequency and its correlation with recognized risk factors. Study was prospective for a five years period; 158 patients, with median age at diagnosis = 13.94 years were followed up by clinical assessment, liver function tests (LFTs), abdominal ultrasound examinations (US) and CFTR tests. In some cases liver biopsy, MRI and elastogramme was performed.

Results: Cystic fibrosis associated liver disease (CFLD) was diagnosed in 51 patients (32.27%), slightly predominance of boys. The disease occurred more frequently in adult patients and among children age 7-14 years, most of cases being diagnosed after 10 years.

Class I and II mutation were present in 56.87% CFLD patients. Meconium ileus was a risk factor (OR=1.12) for developing CFLD, being present in 21% from CFLD patients. Pancreatic insufficiency was strongly associated with LD, certified to be risk factor (OR=1.25).

Conclusion: The frequency of CF associated liver disease is rising. CF children older than 10 year, with severe mutation, history of meconium ileus, pancreatic insufficiency and are more likely predisposed to develop liver disease. In which way the disease evolution or the risk factors control can be influence remain to be determined.

P13.14 Procalcitonin is a specific marker of diabetic complication process

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Introduction: The procalcitonin (PCT) gene, referred to as Calc-1, is located on chromosome 11p15.4 and was sequenced in 1989. The promoter has sites for basal transcription factors but more interestingly, also has sites for Nuclear factor $[\kappa]\beta$ (NF $[\kappa]\beta$) and activator protein -1 (AP-1), factors induced under inflammatory conditions. Diabetes Mellitus is associated with oxidative stress and elevation of advanced glycation end products (AGEs). AGEs are produced by a non-enzymatic, maillard reaction between reducing sugars and either proteins or lipids. AGEs interacts with the receptor for advanced glycation end products (RAGE) and RAGE activation is caused by elevation of the transcriptional factors NF $[\kappa]\beta$ and AP-1. These factors induce PCT gene expression.

Aim: To determine whether serum PCT level is a specific marker in patients with diabetic complications .

Patients: Twenty patients with diabetic foot were studied along with age and sex matched normal non-diabetic subjects (10 males and 10 females) Blood samples were taken for the measurement of serum PCT levels. Serum PCT levels were analyzed by a kryptor analyzer using kryptor-PCT kit designed for KAIA

Results: Serum PCT levels were elevated in diabetic foot patients when compared with those of normal subjects ($p<0.01$).

Conclusions: It revealed a raise in serum PCT levels in patients with diabetic complications. Our study results and experimental design imply PCT as an important mediator during the development of diabetic complications. This algorithmic mechanism was based on genetic expression of PCT. Thus, the results of this study show procalcitonin as a new marker for diabetic complications.

P13.15 Quick evaluation and classification of alpha-Galactosidase A mutants in Anderson Fabry Disease - Prediction of disease relevant mutations

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Fabry disease, a lysosomal storage disorder is based on the deficiency of α -galactosidase A (GLA) activity. Some amino acid exchanges might have only mild effects on enzyme activity reduction. Thus, we developed an in vitro model based on the overexpression of GLA mutants using a HEK293 cell system. Using fluorimetric enzyme activity assay, we are able to determine the activity of mutant enzyme. We tested over 100 mutations with regard to enzyme activity, protein stability and response to the Pharmacological Chaperone (PC) 1-Deoxygalactonojirimycin (DGJ). We raised the data in order to classify the mutations, find hints for the right therapy for the patients and make predictions on the importance of certain amino acids and their positioning inside the molecule by analysing partially novel and described mutations stretching along the whole gene.

P13.16 FBS vs. Osteogenesis Imperfecta. Report of an Iranian Girl with a Private Novel Mutation in GLUT2 gene

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Fanconi-Bickel Syndrome is a very rare hereditary metabolic disease, characterized by hepatomegaly due to glycogen storage, refractory hypophosphatemic rickets, marked growth retardation and proximal renal tubular acidosis. Recurrent bone fractures are one of the hallmark findings. It is a single gene disorder; the responsible gene is belonging to the facilitative glucose transporters family (GLUT2) gene or (SLC2A2). It was mapped to the q26.1-26.3 locus on chromosome 3, encodes the glucose transporter protein 2. This protein is expressed in pancreatic β -cells, hepatocytes, renal tubules, and intestinal mucosa. Several mutations in the GLUT2 gene have been reported in different populations.

Herein we report an Iranian girl with a missed diagnosis of osteogenesis imperfecta. Proband was product of a normal pregnancy, an uneventful delivery, and product of a complex consanguineous marriage. She was referred with the history of frequent fractures, and severe motor delay. Following the case we detected refractory rickets instead of OI, sever growth failure, proximal renal tubulopathy and RTA, and enlarged kidneys by sono, progressive hepatomegaly, and GSD on liver biopsy. Glucose and galactose tolerance test confirmed abnormal carbohydrate metabolism. Diagnosis of FBS was suspected, and treatment with corn starch, Rocartrol, and GH ameliorated her condition satisfactorily.

Molecular analysis on GLUT2 revealed a homozygous private novel mutation in exon 5; it was 15 nucleotide deletion and 7 nucleotide insertion in the point c.685-707 of the gene. It is a frame shift mutation and a premature termination of translation (P.A229QFsX19).

P13.17 FoxP3 expression and regulatory T-cells on earlier and advanced stages of type 1 diabetes mellitus.

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We investigated FoxP3 expression and T-regulatory CD4+ CD25+ cell quantity on different stage of type 1 diabetes mellitus development and in control group. The T-regulatory cell quantity in the group of individuals positive on glucose tolerance test (pGTT) was not different from the control. FoxP3 expression level in this group was twice lower than in control. Among for the first time revealed type 1 diabetes mellitus patients (up to 1 year, early disease stage) FoxP3 expression was approximately as in pGTT group and significantly different from the

control ($P=0.005$). In the same time, T-regulatory cell quantity was decreased for the first time revealed diabetes group in comparison with pGTT group ($P=0.001$). It is interesting that T-regulatory cells were decreased both among patients with remission ($P=0.03$) and without ($P=0.0004$) in comparison with pGTT although their quantity under remission were 1.5 times more than without remission. The FoxP3 expression was changed in this proportion also. During 10 years after type 1 diabetes mellitus onset FoxP3 expression was decreasing and lower than in control ($P=0.0006$). In the group of patients through 6-10 years after disease beginning FoxP3 expression was three times lower than in the group for the first time revealed diabetes. After 10 years of disease the level of FoxP3 expression was increased and was significantly different from the control.

P13.18 THE GENETIC ORIGIN OF GALACTOSEMIA IN BELARUS

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A study of the most common mutations Q188R and K285N in the galactose-1-phosphate uridylyltransferase (GALT) gene from 15 unrelated galactosemia families is reported here. Diagnosis was based on thin layer chromatography for galactosuria/galactosemia, galactose and galactose-1-phosphate determinations and assays of erythrocyte GALT activity. Patients were screened for the common K285N and Q188R transferase gene mutations, using PCR-based assays. All the patients showed markedly above-normal concentrations of galactose and galactose-1-phosphate and less than 10% activity of GALT. According to the laboratory findings K285N and Q188R alleles together represented 64% of detected mutations. We detected 11 alleles carrying the Q188R mutation out of the analyzed 34 (32 % allele frequency). From these, 4 patients were found to be homozygous for the mutation and 3 to be heterozygous, 2 of them were compound heterozygous for the Q188R and the K285N sequence changes. K285N mutation was also identified in 11 alleles (32 % allele frequency), 2 patients appeared to be homozygous. Our results support the idea about Slavic origin of K285N mutation and prove the assumption that the frequency of Q188R decreases from west to east across Europe. It is also supposed that classical galactosemia shows low allelic heterogeneity in Byelorussian patients.

P13.19 Hemochromatosis gene (HFE) variants and risk of iron overload: a meta-analysis

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Purpose: The incomplete phenotypic penetrance of HFE genotypes in relation to hemochromatosis poses a practical problem in the interpretation of the genotyping results by clinicians. We performed meta-analyses of the associations between hemochromatosis genotypes C282Y/C282Y, C282Y/H63D, C282Y/wild type, H63D/H63D, H63D/wild type, versus wild type/wild type and iron status. Methods: MEDLINE search was performed from 1997 to 2008. After reviewing 3572 article titles and evaluating 92 articles in detail, odds ratios (OR) were pooled from 43 study populations using a random-effects model. Potential sources of heterogeneity were also explored. Results: A total of 9,986 cases and 24,852 controls were included in the meta-analysis. The aggregate overall results showed that homozygosity for the C282Y mutation conferred the highest risk for iron overload (pooled OR, 1006.14 [95% confidence interval, 443.21 to 2284.08]) whereas H63D/wild type genotype was associated with the lowest risk of iron overload (OR, 1.68 [95% CI, 1.33 to 2.12]). The subgroup analyses performed showed strong associations between all genotypes and elevated serum ferritin, transferrin saturation, serum iron concentration, hepatic iron score, hepatic iron index, and mobilization of iron in phlebotomy, except for H63D/H63D homozygotes that were strongly associated with elevated hepatic iron index and mobilizable iron by quantitative phlebotomy. Conclusions: The present meta-analysis reliably quantifies the effects of genetic risk on iron overload. It shows an important role for liver biopsy and assessment of mobilizable iron by quantitative phlebotomy in the diagnostic pathway of hereditary hemochromatosis. The results, mainly from case-control studies, cannot necessarily be extrapolated to the general population.

P13.20 Study of mutations in 9 exons of LDLR gene in possible familial hypercholesterolemia patients in a province of Iran

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Background and aim: Familial hypercholesterolemia (FH) is an autosomal dominant disorder caused mainly by mutations in the low-density lipoprotein receptor (LDLR). Until now, the molecular basis of FH has been demonstrated in detail in many populations, but there is still very limited molecular data concerning FH in Iran. The aim of this study was to investigate frequency of LDLR gene mutations in an Iranian population.

Method : in this descriptive -lab based study a total of 50 non-related possible FH subjects from Cheharmahal va Bakhtiari were studies .All samples were tested for presence of LDLR gene mutations in 9 exons of the LDLR gene including 2 , 4 , 6 ,7 , 8 , 9 , 10 , 12 and 14 using SSCP technique. The shifted bands were detected on gels and confirmed by sequencing.

Result: In this study after using SSCP technique and confirmed by sequencing we were found 4 Polymorphisms including 1413 G >A, 1725 C >T , 1773 C >T and 2140 +5G>A in %18 of population studied.

Conclusion: our data indicated that LDLR gene mutations have not contribution to FH in samples studied here. However, we examined for only 9 exons of LDLR gene in only 50 patients, and to determine the role of mutations of this gene in developing FH in Cheharmahal va Bakhtiari province, more FH samples/populations needed to be investigated.

P13.21 A novel mtDNA mutation in a child with Leigh-like syndrome

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 A case of Leigh syndrome with atypical manifestation caused by a novel mtDNA mutation in a 2.5-year-old boy is presented. The delivery at 36-37 weeks of gestation passed with no serious complications. Early motor development was moderately delayed, in 15 months the boy had lurch. In 1.5 years, after a gastrointestinal infection, the unsteadiness increased dramatically. Since this episode the disease has an undulating course with motor deteriorations triggered by common infections. In 'good' periods the boy walks unsteadily though independently and attempts to run. Speech and mental development is delayed but never worsened. Expressive language is poor but receptive language is satisfactory, emotions and behavior are adequate. There were no seizures, vision, hearing or somatic problems. On examination moderate microcephaly (46 cm) was noted. MRI showed symmetric lesion of capsula externa and putamen. Leigh disease was supposed. After SURF1 gene mutations were excluded, a search of mtDNA mutations in blood cells was performed. A novel substitution m.8839G>C in mtATP6 gene was detected in homoplastic state in the child and heteroplastic state in the mother.

We proposed this substitution being pathogenic because the involved mtDNA region is highly conservative and a nucleotide replacement leads to substitution of aminoacid Ala to Pro. In 205 control DNA samples the same substitution was not found.

Thus, this case contributes to clinical variability of Leigh syndrome. Such cases prove the necessity of whole mtDNA sequencing, because very often there is no evidence of causative mutation, if frequent mutations are excluded.

P13.22 First Brazilian case of lysinuric protein intolerance (LPI).

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Lysinuric protein intolerance (LPI) is an autosomal recessive disease, relatively common in Finland. It is caused by defective intracellular transport of cationic amino acids, which leads to inefficient renal tubular reabsorption and intestinal absorption of lysine, arginine and ornithine, considered essential in urea cycle.

It is a multisystemic disease with a variety of clinical symptoms including nausea and vomiting after protein ingestion, hepatosplenomegaly, failure to thrive, muscle weakness. Renal insufficiency, lung involvement and hematological abnormalities are also found.

Mutations in SLC7A7 gene are responsible for this disease, but no genotype-phenotype correlations have been defined.

Treatment is based on low-protein diet and supplementation with oral citrulline.

Our patient is a 2 year-old boy, first child from consanguineous couple. Pregnancy and delivery was uneventful. By one month hepatomegaly was noticed. Invasive diarrhea and recurrent infections started after weaning. He evolved with pulmonary symptoms and chronic respiratory insufficiency by 1y9mo. Laboratorial investigations were: pulmonary biopsy- pulmonary alveolar proteinosis; hepatic biopsy- steatosis and discrete cholestasis; quantitative amino acids analysis- elevated lysine and orotic acid in urine, elevated glutamine in plasma; hyperammonia, elevated chitotriosidase; isoelectric focusing and filipin test in fibroblasts were normal. Molecular analysis of SLC7A7 gene was not done. Presently the patient is alive, 3y5mo, and improved respiratory distress with protein restriction diet.

This is the first Brazilian case of LPI. The diagnosis of LPI is often difficult due to unspecific symptoms with multisystem involvement overlapping with several other metabolic diseases. Molecular analyze of SLC7A7 gene should be performed to confirm the diagnosis.

P13.23 Benefit of inherited metabolic disorders screening in child with cardiomyopathy

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 Cardiomyopathy (Cm), a rare form of cardiac disease in infancy, is receiving increasing attention stimulated by the availability of endocardial biopsy and new forms of therapy.

Through a survey ,we calculated the prevalence inherited metabolic disorders in children with unknown cardiomyopathy .

In accordance with these strict criteria, Our studies involved 87 patient

In seventeen children symptoms were present between ages 10-25 (mean 17) months with general loss of abilities and fallow by cardiac symptoms .

We measured their, lactate, pyruvate, carnitine profile& blood glucose and ketone body. Then we controlled organic acid, amino acid, mitochondrial respiratory chain complex activity in skeletal muscle & acid -alfa - glucosidase activity was measured in infants whom have hypertrophic cardiomyopathy.

Result: The prevalence of metabolic disorders was : Pompe disease (5) ,MPS (7) ,fatty acid - beta - oxidative defect(4)VLCHD, respiratory enzyme deficiency.

Conclusion :Search for inherited Metabolic disorders is better to be performed in all children with cardiomyopathy as the prevalence of metabolic disorders is high in this group and in our country because high incidence of consanguinity marriage .In other hand it may help us for genetic counseling and prenatal diagnosis for families who have affected child .

P13.24 Association between symptoms of depression and anxiety and metabolic syndrome: the modifying effect of C-reactive protein gene (CRP) polymorphisms**

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Depression is associated with the development of the metabolic syndrome (MetS) and both disorders with markers of systemic inflammation, such as C-reactive protein. We examined associations between symptoms of depression and anxiety at age 13-15 years and at age 36 years, and MetS at age 53 years in a large representative British birth cohort. We also investigated whether two CRP polymorphisms (rs1205 and rs3093068) were associated with affective symptoms and the MetS, and whether the risk of the MetS in those with affective symptoms was modified by these CRP gene polymorphisms. Those with depression/anxiety in adolescence (OR=1.30, 95%CI: 0.98, 1.74) and adulthood (OR=1.41, 95%CI: 0.97, 2.45) were more likely to have the MetS. These associations were stronger in women than in men. Although CRP SNPs were not associated with affective status or the MetS, the association of adolescent emotional problems with the MetS

was stronger in those who were homozygous for the major allele (C) of rs1205 (OR=1.83, 95%CI: 1.17, 2.86) than in carriers of the T allele (OR=1.01, 95%CI: 0.66, 1.55) (p=0.05 for gene by affective status interaction). Adolescent-onset depression and anxiety may play an important role in the MetS risk later in life, particularly in those homozygous for the major allele of CRP rs1205. These findings may highlight new ways of identifying depressed people at high risk of developing the MetS, which is of great importance for the treatment and clinical management of depressive patients.

P13.25 Proteomics reveals new insights into the causes of hyperammonemia in methylmalonic acidemia

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Methylmalonic Acidemia (MMA) is an inborn error of metabolism that is caused by a deficiency in the mitochondrial enzyme methylmalonyl-CoA mutase. Clinically, patients with MMA exhibit elevated levels of methylmalonic acid in their plasma and urine. During periods of illness, MMA patients can experience life-threatening metabolic instability with intermittent bouts of hyperammonemia; the underlying mechanisms of the pathology of these episodes are not well understood. Presently, the inhibition of N-acetylglutamate synthetase (NAGS) by propionyl-CoA, a metabolite elevated in MMA, is thought to indirectly decrease carbamoyl phosphate synthetase I (CPS1) activity causing hyperammonemia. To investigate possible mechanism(s) of pathology, we performed proteomic analysis of liver extracts from a mouse model of MMA using two dimensional fluorescence difference in-gel electrophoresis (DIGE) and quantitative analysis using iTRAQ labeling and tandem mass spectrometry. Proteomics of the liver from MMA mice revealed changes in the expression of the urea cycle (UC) enzymes CPS1, ornithine transcarbamylase (OTC), argininosuccinate synthetase 1 (ASS1), argininosuccinate lyase and arginase compared to controls. MMA mice have decreased levels of plasma ornithine, but normal levels of urine orotate, plasma arginine and plasma citrulline. Limited CPS1 activity due to NAGS inhibition is consistent with low to normal urinary orotate levels. Since deficiencies in CPS1 and OTC can cause hyperammonemia, we propose that the changes in the expression urea cycle enzymes contribute to the occurrence of hyperammonemia.

P13.26 Analysis of *MUT* gene mutations in Turkish patients with methylmalonic acidemia using resequencing microarrays: identification of fourteen novel mutations

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Methylmalonic acidemia (MMA) is an autosomal recessive inborn error of the organic acid metabolism. The condition is resulted from functional defects in the methylmalonyl-CoA mutase (MCM) enzyme or disorders of cobalamin metabolism. Patients with methylmalonic acidemia have increased levels of methylmalonic acid in blood and urine and can suffer from extreme acidosis. The human MCM gene (*MUT*) is located on chromosome 6 and comprised of 13 exons spanning over 35 kb.

In this study, 34 Turkish patients diagnosed with methylmalonic acidemia were screened for mutations using Affymetrix resequencing microarrays and all the detected mutations were confirmed by direct sequencing. As a consequence of direct sequencing, mutations of 29 patients were detected. The resulted mutations consisted of twenty one missense mutations, six nonsense mutations, one splicing mutation, one insertion mutation, and two deletion mutations, 21 of 29 patients were homozygous for the both mutant alleles. DNA sequencing analysis revealed fourteen novel mutations. The most common of the missense mutations were P615T mutation in exon 11, accounting for nearly 43% of the all missense mutations and nearly 30% of the all the mutations.

P13.27 Mitochondrial disorders due to Mitochondrial DNA mutations on respiratory chain in Bulgaria

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Mitochondrial disorders are rare disorders due to mitochondrial DNA or DNA mutations. By integral approach with clinical, biochemical methods, EEG, EMG, KAT, MRI and MT-DNA investigation in peripheral intravenous blood and skin fibroblast, sequenced PSR-SBT method of mtDNA regions were diagnosed various mitochondrial disorders. The aim of our work were to evaluate the received data, to make genotype/phenotype correlations and to proceed the results of treatment. There were diagnosed 40 children with mitochondrial DNA disorders of Complex I, Complex III and Complex V. All found mutation of Mt-DNA were new, not registered to this time in international register for mitochondrial DNA mutation. All these mutation were unknown function. We discovered 3, ATP synthase 8 deficiency patients. The clinical presentation of the found mutations were the same as similar mutations described in the literature. For treatment of our children were used low protein diet, very high doses vitamins, L-carnitine. The best results were received in patients with Complex 1 deficiency, in the other there were decrease the convulsions, but no significant improvement in mental development and neurologic symptomatics. On the parents were offered genetic consultation and possibility for prenatal diagnosis.

P13.28 Novel phenotype associated to mutations in the *SDHD* gene resulting in complex II deficiency

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Defects of the mitochondrial respiratory complex II (succinate dehydrogenase, SDH) are extremely rare. Of the four nuclear encoded proteins composing complex II, only mutations in the 70kDa Flavoprotein (SDHA) and the recently identified complex II assembly factor (SDHAF1) have been found to be causative of a mitochondrial disorder. Mutations in the other three subunits (SDHB, SDHC, SDHD) and the second assembly factor (SDHAF2) have so far only been associated with hereditary paragangliomas and phaeochromocytomas.

Here we report the first ever described case of a mutated subunit other than SDHA and SDHAF1 causing a mitochondrial disorder with an isolated complex II deficiency. The patient showed a progressive retardation after the age of six months evolving in a severe encephalopathy with choreo-athetotic movements, optic atrophy and intractable myoclonic seizures at the age of eight years. MRI and MRS were normal at the age of ten months. Complex II had a residual activity of 10% in muscle. Analysis by comparative 2D BN-PAGE following MALDI-TOF MS demonstrated the absence of SDHA. Further western blot analysis confirmed reduced expression of SDHA and SDHB in skeletal muscle. Molecular genetic analysis of SDHA, SDHB, SDHC, SDHD, SDHAF1 and SDHAF2 revealed compound heterozygosity for two mutations located in a transmembrane domain of SDHD.

We conclude that the mutations of the SDHD-gene result in abolition of its protein to integrate into the membrane impairing all other subunits to assemble into a functional SDH-complex and are subjected to degradation.

P13.29 Mitochondrial ND5 mutations mimicking brainstem tectal glioma

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We report MRI-periaqueductal T2-hypersignal suggestive of tectal glioma in three unrelated children with reduced vision and normal mental development. Increased CSF lactate and optic atrophy in the first case suggested mitochondrial dysfunction. Muscle biopsy revealed

Complex-I deficiency. A heteroplasmic mt-ND5 mutation was found (m.13513G>A). The second case presented with similar clinico-radiological features, Complex-I deficiency and the same heteroplasmic mutation. The third case had visual disturbance without optic atrophy, normal muscle enzyme activities, but a heteroplasmic mt-ND5 mutation (m.13514A>G). Even in absence of optic atrophy, mental retardation or multiorgan dysfunction, the combination of visual disturbance and periaqueductal T2-hypersignal should prompt the search for mitochondrial-DNA mutation.

P13.30 MITOCHONDRIAL MYOPATHY OF CHILDHOOD ASSOCIATED WITH MITOCHONDRIAL DNA DEPLETION

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Mitochondrial DNA depletion syndromes (MDSs) form a group of autosomal recessive disorders characterized by profoundly decreased mitochondrial DNA copy numbers in affected tissues. Three main clinical presentations are known: myopathic, encephalomyopathic and hepatocerebral. The first is associated with mutations in thymidine kinase 2 (TK2) and p53-induced ribonucleotide reductase B subunit (RRM2B) genes. This study aimed at the description of the molecular diagnosis of 1 Egyptian pediatric patient presented to the Cairo University Pediatric Hospital (CUPH) with the clinical suspicion of mitochondrial myopathy. Results of histochemical staining of the muscle biopsy specimens showed Cytochrome Oxidase negative fibers. Biochemical analysis of the muscle homogenate revealed absence of Complex I and Complex IV activity compared to age matched controls. Quantitative radioactive Southern blot analysis showed reduction of the mitochondrial/nuclear (mt/n) DNA ratio (\approx 30 % of aged-matched controls). Sequencing of the TK2 gene revealed no sequence variation. Targeted molecular diagnosis based on the biochemical analysis of the respiratory chain enzymes makes the molecular evaluation of pediatric mitochondrial disorders much easier. Involvement of other nuclear genes rather than TK2 gene in the pathogenesis of the myopathic form of MDS should be considered.

P13.31 New MPV17 mutations in a child with mitochondrial hepatoencephalopathy. Case report

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Mitochondrial hepatoencephalopathies comprise the group of mitochondrial cytopathies that affect children and young adults. Most of them characterized by severe reduction mtDNA copy number (mtDNA depletion). Several genes recently have been identified to cause mtDNA depletion syndrome (MDS): TK2, SUCLA2, DGUOK, POLG, RRM2B and MPV17. Here we report a case of 3 year old girl with hepatoencephalopathy and mutations in MPV17 gene. First symptoms manifested at 3 months of age by failure to thrive, vomiting and dry skin. At 7 months she developed ophthalmoplegia and episodes of subfebrile temperature. MRI at 12 months showed meningoencephalic lesions. Later she developed hepatopathy, myopathy, hypoglycemia and ketoacidosis. At the age of 2 years the girl was able to sit, stand with support, had speech and normal mental development. After the episode of hypoglycemic coma she developed severe myopathy and areflexia. Muscle biopsy showed red ragged fibers and increase number of paracrystalline mitochondria. 6 months later patient's condition worse a lot. She presented with dysarthria, ataxia, polyneuropathy, cardiomyopathy and hyperextention of the knee joints. The patient was diagnosed with hepatic cirrosis at the age of 3 years. We made full sequence of genes POLG and MPV17 and revealed two novel heterozygous mutations in the MPV17 gene: c.185delT and c. 121C>T (R41W). To our knowledge about 30 patients with MPV17 gene mutations have been described in the literature. We consider MPV17 gene analysis to be done for MDS patients, especially with slowly progressive liver disease.

P13.32 Application of MLPA technique in detection of single large-scale mitochondrial DNA deletions.

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Single large-scale mitochondrial DNA deletions varying in size and location, occur mainly as sporadic cases and are usually associated with known deletion syndromes (KSS, PS and PEO). Characteristic clinical features include: progressive external ophthalmoplegia, generalized muscle weakness with difficulties in swallowing and articulation, short stature, deafness, conduct disturbances, delayed puberty, and endocrine dysfunction. The most common deletion responsible for almost 30% of deletion syndromes, contains 4977 bp and is located between nucleotides m.8469 and m.13147.

The aim of the study was to examine the utility of the MLPA technique in the detection of mtDNA deletions. The SALSA MLPA KIT P125 Mitochondria (MRC-Holland) contains 31 probes for different mtDNA sequences, and 1 mutation-specific probe for the frequent point substitution m.3243A>G.

Fifteen patients with mitochondrial cytopathies (including 5 KSS), and seven controls (5 healthy subjects, and 2 patients with known m.3243A>G mutation) were enrolled into our study.

Various heteroplasmic deletions spanning regions: m.9169_14174 (ATP6, MTCOIII, MTND3 - MTND6 genes), and m.10922_15765 (MTND4 - MTND6, CYB genes) were identified in two patients. The last one was confirmed by Southern hybridization. In other six children no hybridization of single probes to sequences of MTND2, MTND4, MTND6 and MTATP8 genes was found. The presence of m.3243A>G mutation was confirmed in both control cases, whereas remaining changes require verification by other methods.

MLPA technique seems to be a useful tool in identification of heteroplasmic large-scale mtDNA deletions.

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P13.33 The diagnosis of Mucopolysaccharidosis over 11year: the Egyptian experience.

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Mucopolysaccharidosis is a group of inherited metabolic disorders caused by deficiency of specific enzymes needed for the metabolism of mucopolysaccharides called glycosaminoglycans (GAGs) which leads to their accumulation in lysosomes of different tissues and organs.

Aim: Laboratory diagnosis of MPS among the clinically suspected cases & to find out the distribution and frequency of each type of MPS among our population.

Subjects and Method: The present study included 1041 patients referred during the last 11 years for the diagnosis or exclusion of MPS. Each patient was subjected to quantitative determination of urinary GAGS. Cases with high concentration were subjected to extraction of GAGS from urine followed by electrophoretic separation. Activity of the specific enzymes according to the abnormal pattern was assayed fluorometrically.

Results: Among 1041 patients screened for MPS, 540 had elevated GAGS in urine. Using the electrophoretic separation of GAGS, 319 cases proved to have MPS. The specific enzyme assay for the 319 positive cases revealed the following distribution: MPS type I n = 81, MPS type II n = 51, MPS type III n = 45, MPS type IV n = 65 and MPS type VI n = 74.

Conclusion: Quantitative determination of GAGS in urine is a simple procedure to select cases for its electrophoretic separation. However, enzymatic assay is mandatory to confirm the MPS types. The commonest MPS disorders among the diagnosed cases in this study were MPS type I and type VI followed by type IV.

P13.34 Mucopolysaccharidosis Type I: Outcome of an Early Diagnosis and Treatment of two Saudi Siblings

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Here, we present an eleven and five years old Saudi siblings with Mucopolysaccharidosis type I. They are homozygous for P533R mutation. The first one was diagnosed at 14 months and the younger

one at birth. The family history is significant for an older affected sister who died at 15 years of age of cardiopulmonary arrest. Enzyme replacement therapy (Aldurazyme 1mg/kg) was commenced on both of them in a weekly base at 3 1/2 years and 9 months of age respectively. There was significant decrease in glycosaminoglycans (GAGs) excretion within the first three month of treatment. Both of the siblings had achieved a normal growth velocity. Their cardiac involvement remained stable with a normal cardiac function. The first sibling continued to have repeated episodes of otitis media. He has sustained hearing impairment, speech delay with learning difficulty. The younger sibling had no further ear infection following the treatment. Her speech and cognitive function were normal. Progressive skeletal deformities mainly of the spine were documented on the first child. On the hand, the younger child had a mild skeletal involvement with maintaining of normal joints mobility. This data emphasized the importance of an early diagnosis and treatment of such condition. A detail description of the clinical data and outcome of both siblings in addition to literature review will be presented.

P13.35 Mucopolysaccharidosis type I in Belarus: clinical characterization of 7 patients

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The assessment of wide variation of clinical presentation in patients with mucopolysaccharidosis type I (MPS I), dynamic of the disease progression is directed toward the early diagnosis of this rare lysosomal storage disorder.

We present here the clinical observation of 6 cases with Hurler (MPS IH) and 1 patient with Hurler/Scheie (MPS IH/S) 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.

MPS IH. Median age at diagnosis in 3 males and 3 females with MPS IH was 1,87 years (range: 0,5-5 years), median age at onset of symptoms was 0,25 years (range: at birth - 1 year). Interval from symptom onset to disease diagnosis was 1,6 years (range: 0,5-4 years).

All patients (100%) presented severe mental retardation, "coarse" facial features, joint stiffness and contractures. Over 83,3% of our patients showed macrocephaly, umbilical hernia, corneal clouding, hepatosplenomegaly and cardiac abnormalities.

MPS IH/S. 1 male with MPS IH/S had "coarse" facial features, normal intelligence, joint stiffness, contractures and claw hands, hepatomegaly and moderate mitral regurgitation. Median age at diagnosis was 3,8 years, median age at onset of symptoms was 3 years old. Conclusions: Patients with MPS I are very heterogeneous group including three clinically delineated types. The detailed analysis of clinical data provides the evidence-based approaches of the formation high-risk groups of patients both severe Hurler syndrome and mild types Hurler/Scheie and Scheie and contributes to the early clinical diagnostics development.

P13.36 Identification of novel candidate genes for non-alcoholic fatty liver disease (NAFLD) using a bioinformatic approach

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Aim: This project applies a bioinformatic approach to identify potential new candidate genes for non-alcoholic fatty liver disease (NAFLD).

Methods: Text-mining in PubMed and OMIM for genes related to liver disease, visceral obesity, and waist-circumference and subsequent protein-protein interaction analysis were used to identify a small isolated interactome containing genes highly expressed in the liver. This interactome included *EHHADH*, *ECHS1*, *HADHA*, *HADHB*, and *ACADL* which are involved in the mitochondrial fatty acid β -oxidation. Twenty-one tagSNPs (HapMap, CEU, $R^2 > 0.8$) capture all common variation in the 5 genes. All tagSNPs were genotyped using KASPar® and

analyzed for association with surrogate measures of NAFLD (obesity, waist, HOMA-IR, plasma glucose, and serum triglycerides) in 6,514 Danes from the population-based Inter99 cohort.

Results: Four SNPs in *EHHADH* showed an association with increased fasting serum insulin and HOMA-IR levels. The G-allele of rs11101721 in *ECHS1* associated with obesity ($OR_{add} = 1.33(1.06-1.68)$, $p_{add} = 0.02$). Quantitative trait analysis showed an increase in BMI with a per allele effect of 1.5 kg/m² [0.7;2.3], $p_{add} = 0.0005$. This effect was only seen in individuals with impaired glucose regulation (IGR), and hence an interaction between rs11101721 and glucose-tolerance status with effect on BMI was shown ($p_{int} = 0.002$). The T-allele of rs892447 in *HADHA*/*HADHB* associated with increased fasting serum insulin ($\beta = 4\%[8;0.3]$, $p_{add} = 0.04$) and HOMA-IR ($\beta = 5\%[10;1.0]$, $p_{add} = 0.02$) among IGR individuals. Three SNPs in *ACADL* associated with obesity measures.

Conclusion: By using a bioinformatic approach, variation in five candidate genes was found to be associated with several surrogate measures of NAFLD.

P13.37 Comparative genotyping of obese and lean children in Belarus: preliminary results

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Last year started the project concerning the investigations of factor-predictors childhood obesity in belarussian population. 83 obese children from 3 groups: 0,5-8 years old (BMI 20-35 kg/m²), 9-12 years old (BMI 24-35 kg/m²), 13-17 years old (BMI 25-41 kg/m²) and 83 lean control children (1-16 years old) were genotyped using PCR-RFLP analysis of polymorphic alleles of Gln223Arg (A/G) leptin receptor (LEPR) gene, -174G/C interleukin 6 (IL-6), -308 A/G tumor necrosis factor- α (TNF- α), -23/HphIA/T insulin (INS) gene. No significant differences in genotype and allele frequencies were found.

Integrated assessment of metabolic and neuroendocrine status of obese children as well as genotypes of their parents is under investigations.

| Age group and BMI | n | Ins -23HphI (%) | | | LEPR (%) | | | TNF α (%) | | | IL-6 (%) | | |
|----------------------------|----|-----------------|------|------|----------|------|--------|------------------|------|------|----------|------|------|
| | | AA | AT | TT | AA(QQ) | AG | GG(RR) | AA | AG | GG | GG | GC | CC |
| 0-8 >20kg/m ² | 39 | 71,8 | 20,5 | 7,7 | 23,1 | 61,5 | 15,4 | 0,0 | 29,7 | 70,3 | 29,3 | 58,5 | 12,2 |
| 9-12 >24kg/m ² | 27 | 77,8 | 14,8 | 7,4 | 24,1 | 69,0 | 6,9 | 0,0 | 24,0 | 76,0 | 37,0 | 51,9 | 11,1 |
| 13-16 >25kg/m ² | 17 | 35,3 | 52,9 | 11,8 | 33,3 | 61,1 | 5,6 | 0,0 | 18,8 | 81,3 | 44,4 | 44,4 | 11,1 |
| All obese | 83 | 66,3 | 25,3 | 8,4 | 25,6 | 64,0 | 10,5 | 0,0 | 25,6 | 74,4 | 34,9 | 53,5 | 11,6 |
| girls >20kg/m ² | 34 | 70,6 | 17,6 | 11,8 | 28,6 | 65,7 | 5,7 | 0,0 | 29,0 | 71,0 | 31,4 | 60,0 | 8,6 |
| boys >20kg/m ² | 49 | 62,5 | 31,3 | 6,3 | 24,0 | 66,0 | 10,0 | 0,0 | 25,5 | 74,5 | 42,0 | 46,0 | 12,0 |
| Controls | 83 | 61,9 | 34,5 | 3,6 | 19,1 | 62,9 | 18,0 | 0,0 | 28,6 | 71,4 | 34,5 | 47,1 | 18,4 |

P13.38 Resistin gene (RETN) promoter polymorphism and serum resistin level in patient with abdominal obesity

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Resistin is a member of a class of cysteine-rich proteins and may present an important link between obesity and insulin resistance. OBJECTIVES: (1) to compare the frequency of *RETN* polymorphism -180C>G (rs1862513) of the resistin gene in patients with abdominal obesity and in control group. (2) to study an effect of particular genotypes on serum resistin level and indicators of lipid metabolism. STUDY POPULATION: 130 males and 328 females with abdominal obesity aged 30-55 years old. Control group included 110 children and adolescent. METHODS: Serum resistin was measured using an enzyme-linked immunosorbent assay kit, indicators of lipid metabolism were evaluated according to standard protocols. Genotyping was performed by PCR-RFLP. RESULTS: In patients with abdominal obesity, the frequency of C-allele and G-allele was 0.71 and 0.29, respectively. There was no difference in both CC, CG, and GG genotype distribution and in rates of C- and G-alleles of resistin gene between the studied groups (0.7 and 0.3 respectively, $p > 0.05$). No difference was found in serum resistin level, in waist circle and in BMI between patients carrying different *RETN* genotypes. However LDL-C levels was higher in patients carrying

CC-genotype compared to patients carrying CG-genotype (4.06 ± 0.08 mmol/l and 3.8 ± 0.08 mmol/l respectively, ($p < 0.05$)). CONCLUSIONS: We did not reveal any difference in serum resistin level in patients with abdominal obesity carrying different resistin gene genotypes. LDL-C level was higher in patients carrying CC-genotype.

P13.39 Identification of L194R, novel mutation in phenylalanine hydroxylase gene (PAH) in Iranian population

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Phenylketonuria (PKU), the most common disorder of amino acid metabolism, is an autosomal recessive disease caused by mutations in the phenylalanine hydroxylase (PAH) gene. The incidence of PKU in Iran has been estimated at 1 in 3627 live births. To date, hundreds of mutations leading to PKU have been identified in the PAH gene. The spectrum of these mutations differs among different populations. In the present study, a novel mutation has been identified- during the mutational screening of the PAH gene in 150 Iranian families- in the human phenylalanine hydroxylase gene of a patient with classical PKU. It is a single base transversion of T to G at the second base of codon 194 in exon 6 of PAH gene. This mutation results in a Leu to Arg change in the catalytic domain of the protein.

P13.40 Maternal phenylketonuria in Russia

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Background

Charles Dent [1957] was first who drew attention to the maternal Phenylketonuria as syndrome. He described three children with mental retardation without PKU which were born from PKU women. There was described many different antenatal effects of high blood phenylalanine level on the foetus. But actually the knowledge about such children health and development is limited. We wanted to determine the problem of maternal PKU in Russia.

Method

Were interviewed the leaders of regional genetic service in Russia. The questionnaire was developed and included information about:

- Regional medical statistic data (for local Russian territory)
- Reproductive activities of registered PKU women
- Local healthcare regulation

Materials

In October 2009 we distributed 47 questionnaires, 17 questionnaires were retrieved till January 2010.

Screening period ranged from 40 till 12 years for different regions.

Total number of PKU reproductive age women has reached 206. There were registered 33 pregnancies, were born 22 children (only 13 women received strong service and metabolic control during pregnancy).

Discussion

The information we received shows the low (40%) level of dispensary coverage of women identified by screening and insufficient maintenance of metabolic control (59%) within pregnancy. We need continued research for state and local recommendations developing for management PKU women.

P13.41 Mutational analysis of phenylalanine hydroxylase gene in Slovenian patients with phenylketonuria: a preliminary report

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Phenylketonuria (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 1 in 8000. Currently, Slovenian national register of PKU patients contains more than 130 patients. Most of them are planned to be included into

our mutational analysis of PAH gene in Slovenia. Here we report a molecular characterization of the first 20 patients. The entire coding region of the PAH gene spanning 13 exons was PCR amplified and analysed 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 100%. We have identified 13 different disease-causing mutations, three of them novel: p.Arg157Ser, p.Leu15GlnfsX24 and c.845-2A>G. All patients were found to be compound heterozygotes. The single most frequent mutation in Slovenian PKU population is p.R408W in exon 12 (approximately 27.5%), which is in concordance with previous European and regional studies of PAH gene.

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.

P13.42 The role of carrier enzyme systems in the clinical polymorphism of Phenylketonuria

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Phenylketonuria (PKU) is an inborn poly-enzymatic multi-system pathology of the metabolism, its primary block being the Phenylalanine (Phe) hydroxylation that leads to severe mental retardation with clinical and biochemical polymorphism.

Methods: 30 PKU children were investigated for the level of free amino acids in blood and urine determined through liquid chromatography on amino acid analyzer Kovo AAA339, Czech.

The analysis of amino acids concentrations grouped according to the enzyme carrier systems (L-, A/ASC-, X_{AG}-, y⁻, Pro-, β -, Gly-) deduced the decreasing of their function in blood and at the kidney level with increasing tendency only of the carrier system of Gly: and significant statistically ($p < 0.001$) increasing of the L: carrier system function in blood in PKU patients. As the result, in addition to the Phe disorders, the troubles of the Met and Trp metabolism, ornithinic cycle were appreciated.

In metabolic disorders of PKU have been appreciated many important pathways as: the decreasing of excitatory of CNS amino acids level (Asp, $p < 0.01$, Glu $p < 0.001$, His $p < 0.001$), increased urinary excretion of stabilizing CNS amino acids (Tau, Tyr, Trp $p < 0.01$), also the tendency of increasing of inhibitory amino acids quantity (Gly, GABA). At the consequences, the inhibitory processes in CNS are prevalent, evaluating with slow mental development.

Conclusion: The effectiveness of the PKU treatment increases if combining the low Phe diet with a metabolic correction of enzyme carrier systems disfunctions.

P13.43

Variable Number Tandem Repeat Marker for Carrier Detection of Pku In Central Iran

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Phenylketonuria (PKU) is the most common error in the metabolism of amino acids and an important genetic disorder, which- if untreated- results in mental retardation. The incidence of PKU among the Caucasians in general is 1 in 10000. The highest prevalence for PKU has been reported from Iran and its neighboring countries (around 1 in 4000). This is due to the high prevalence of consanguineous marriages in this region. Due to the large number of PKU-causing mutations in the phenylalanine hydroxylase gene, segregation analysis of the Variable Number Tandem Repeat (VNTR) polymorphic marker associated with this gene is applied in carrier detection of PKU. Level of informativeness of this marker in carrier detection of PKU in a population depends on the number of its alleles and distribution pattern of these alleles in the population. Considering the population heterogeneity in Iran, we investigated allelic frequencies of this marker in the province of Yazd (central Iran). 33 mutant and normal chromosomes from 9 unrelated

PKU families were studied by PCR and gel electrophoresis. 5 VNTR alleles were identified. All of them were present in normal chromosomes but only 4 of them were present in mutant chromosomes. One of these 4 alleles accounted for 78% of mutant chromosomes. Pattern of distribution of alleles in normal and mutant chromosomes were markedly different (20%, 20%, 33%, 13.5%, 13.5%). Polymorphic Information Content (PIC) of this marker was 63%, which indicates it as a suitable marker for carrier detection of PKU in the population under study.

P13.44 Tissue-specific activity of a new phenylalanine hydroxylase gene enhancer

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Phenylketonuria (PKU) is metabolic disorder caused by mutations in the phenylalanine hydroxylase gene (PAH). Although PAH genotype is the main determinant of the severity of patient's phenotype, inconsistencies were reported. To explain phenotypic differences between patients with the same genotype, we analyzed a potential transcription regulatory sequence from PAH gene intron 8 (I8).

Electromobility shift assays (EMSA) confirmed interaction of I8 with nuclear extracts from both, HepG2 and K562 cell lines. Competition and supershift EMSA experiments revealed that GATA-1 transcription factor binds to I8, while South-Western blot verified that the protein complex bound to I8 contains GATA-1 among other tissue-specific transcription factors.

Furthermore, we analysed activity of pBLCAT5_I8 construct in functional *in vitro* assays. Analysed construct showed 3.8 times higher reporter activity in comparison to basal activity of pBLCAT5 in HepG2 cell line. Student's t-test confirmed the statistical significance of this finding ($p=0.04$). In contrast, the same construct expressed 0.8 times lower activity in K562 cell line, demonstrating importance of characteristic hepatoma cell line transcriptional factors for I8 activity as an enhancer.

We concluded that PAH intron 8 contains a new transcriptional regulatory element which binds GATA-1 transcription factor and acts as an enhancer specifically in the hepatoma cell lines. Revelation of this modulated PAH expression could have an impact on the better understanding of PKU phenotype complexity. In addition, it is the first time that an enhancer has been identified in the PAH intron. Thus, presented data could unlock new area for analysis of PAH gene expression.

P13.45 The analysis of genotype and phenotype of Estonian phenylketonuria patients for finding out potentially BH_4 -responsive forms

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Introduction: The clinical phenotype of phenylketonuria (PKU) and potential tetrahydrobiopterin (BH_4) - responsiveness depends on residual phenylalanine hydroxylase (PAH) activity and can be predicted by patient's genotype. The aim of the study was to analyze the genotype-phenotype data of present Estonian PKU patients, to find out the prevalence of potentially BH_4 -responsive PKU forms, and in order to suggest the inclusion of the BH_4 loading test in the PKU diagnostic protocol in newborn screening program.

Material and Methods: The clinical data about 75 PKU patients in Estonia is partly available since 1979 and fully available since 1993 when newborn screening for PKU was started. The genotypic data was available in 72 of them.

Results: Forty eight of patients (67%) were homozygous for R408W mutation in the PAH gene and had clinically classical PKU. Patients with R408W/IVS12nt1, R408W/R252W, R408W/P281L, R408W/E221D222fsdelAG and R158Q/IVS12nt1 genotype showed classical PKU, except one moderate PKU patient with genotype R408W/R252W. Patients with R408W/I306V genotype had mild and with R408W/L48S, R408W/S349P genotype moderate PKU. Two classical PKU patients had R408W/R261Q genotype with many complaints in spite of dietary treatment. In one of them we have started BH_4 treatment with good response.

Conclusion: Seven (9.3%) previously diagnosed Estonian PKU patients with R408W/I306V, R408W/L48S and R408W/R261Q genotype from mild to classical PKU form, can be good candidates for the BH_4 treatment after positive results on loading test. Therefore, in the future it is also reasonable to perform a BH_4 loading test in all PKU patients after the detection by newborn screening.

P13.46 Completeness of mutation spectrum among Slovak PKU patients

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Phenylketonuria (PKU) is a heterogenous 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. Thirty-two mutations have been identified so far, and these make up 88 % of all PKU alleles. The aim of this work was to carry out a complete mutation analysis in a sample of 75 unrelated PKU patients with 1, or with no known mutation. HRM (High Resolution Melting) and MLPA (Multiplex Ligation-dependent Probe Amplification) methods were applied to screen all 13 exons of the PAH gene. Amplicons with differing melting curves were subsequently sequenced to characterize the DNA variants. Sequencing revealed several different substitution mutations and polymorphisms. MLPA analysis was applied for the detection of large deletions and gene duplications, not previously found in the Slovak population. Additionally, the corresponding frequencies for all mutations were estimated, and their classification according to phenotypic categories of PKU was identified. Detailed results will be presented in our poster.

P13.47 Detection of a novel mutation in the GAA gene in an Iranian child suffering glycogen storage disease type II

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Glycogen storage disease II (GSDII or pompe disease, OMIM # 232300), is a hereditary lysosomal disorder with an autosomal recessive mode. Mutations in the GAA gene on the chromosome 17q25.2-q25.3 are usually leading to the absent activity of the acid alpha-glucosidase (acid maltase, GAA, OMIM *606800, EC 3.1.26.2), which results in impaired degradation and subsequent accumulation of glycogen within the lysosomes. The pompe disease is classified into the classical infantile-onset and the late-onset. The late-onset form is subdivided in the childhood, juvenile and adult-onset; thereby the classification is based on the age of onset, organ involvement, severity, and the rate of progression.

In the present report, the molecular analysis of the entire coding region and the flanking introns of the GAA gene were performed in an Iranian infant suspected with pompe disease regarding to the classical symptoms as hypotonia, cardiomegaly, muscle weakness and acid alpha-glucosidase deficiency. According to the human genome mutation database (www.hgmd.org), we found in the exon 15 of the GAA gene a novel single base insertion (insA) at codon 691 that leads to a premature stop codon at codon 736. To validate the molecular analysis from the infant, the parents were subjected to sequencing of GAA gene. Both the mother and the father were heterozygote for the same mutation. The detected mutation in the GAA gene may cause the disease, which is in accordance with the measured deficient acid alpha-glucosidase activity and other clinical symptoms in the proband.

P13.48 The effect of lactose intolerance and lactose intake on metabolic traits

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Department of Surgery, Sestre milosrdnice University Hospital, Zagreb, Croatia. Lactose intolerance is a condition where one is unable to metabolise lactose and it is almost completely determined by a single nucleotide polymorphism (rs4988235) on the chromosome 2q21.3. The condition affects the majority of the adult human population and is associated with a range of health problems. We want to investigate the effects of lactose consumption on seventeen classical biochemical traits in individuals with different genotype at rs4988235 locus.

Individuals from three populations (Vis and Korcula, Croatia, and Orkney, Scotland; N=2935) were studied, for whom a large number of metabolic traits was available. They were typed at C/T₋₁₃₉₁₀ locus (rs4988235), and lactase persistence phenotype was inferred from the genotype: TT and TC were considered lactose tolerant. Lactose intake was estimated from food frequency questionnaires. Linear regression was used to investigate the effects of lactose intake, lactose tolerance and their interaction on numerous metabolic traits.

In the islands of Korcula and Vis there were 42% and 50% of lactose tolerant individuals, respectively, but as much as 96% in Orkney. The strongest association was found in Vis between rs4988235 and tissue plasminogen activator, tPA, (p-value=0.0004), but association did not replicate in Korcula (tPA not measured) or Orkney (too few intolerant individuals). Significant association was found for lactose intake and HDL in Vis (p-value=0.031), and the association was replicated in Korcula (p-value=0.047).

Lactose tolerance, intake and their interaction seem to affect metabolic traits. Better understanding of this association might have implication in health recommendations.

P13.49 A new UKGTN (UK Genetic Testing Network) service for DHC7 mutation analysis in Smith Lemli Opitz syndrome

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Smith Lemli Opitz syndrome is an autosomal recessive metabolic disorder causing multiple congenital malformations; there is continuum of severity from intrauterine lethality to mild dysmorphism/mental impairment. It results from deficiency of 7-dehydrocholesterol reductase, which catalyses the last step of endogenous cholesterol synthesis, due to pathogenic mutations in the *DHCR7* gene.

The incidence of SLOS in the UK is estimated at 1/15,000 - 1/60,000 births. The carrier frequency may be as high as 3% in Caucasian populations. The incidence of SLOS is lower than expected from carrier rates from mutation studies. This discrepancy may be due to under-diagnosis and lethality of severe cases *in utero*.

The local Biochemical Genetics service has offered SLOS testing by quantitation of 7-DHC since 1995, hence molecular genetic analysis of *DHCR7* builds upon existing local expertise and clinical interest to provide a comprehensive service.

The drivers for developing the molecular service were: 1) biochemical carrier testing on fibroblasts requires cholesterol-free culture media which is challenging and not readily available, 2) clarification where the biochemical test result is equivocal or normal but the phenotype is strongly suggestive of SLOS, 3) testing of parents for retrospective diagnosis of a deceased child or fetus where no other material is available.

We present results from patients tested to date (8 proband referrals, 3 sets of parents at the time of the abstract): 6/8 patients carry at least one copy of the common c.964-1G>C mutation, 9 other different mutations have been detected in these patients, including one apparently novel nonsense variant.

P13.50 Studying of efficiency valproic acid treatment of patients with spinal muscular atrophy respective of CYP2C9 and CYP2C19 polymorphism

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Spinal muscular atrophy (SMA) is a severe autosomal recessive neuromuscular disorder. Clinical trials on SMA treatment with valproic acid

(VA) preparations are in progress in our institute as well as in many other Centers. Detoxification of VA and its major metabolic products is known to be carried out by cytochrome P450 enzyme system, with special emphasis on cytochromes CYP2C9 and CYP2C19. Analysis of the relevant genes polymorphisms (430C>T & 1075A>C for CYP2C9) (681G>A for CYP2C19) and their association with efficiency of SMA treatment was a major goal of the present study. PCR-RFLP analysis was carried out in 35 SMA patients and in 210 individuals of the control group. The genotype and allele frequencies for both studied genes were also quite comparable in the patient and control groups. No correlation of genotype and allele frequencies for CYP2C9 and CYP2C19 polymorphisms respective of VA treatment efficacy were found.

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P13.51 Screening for metabolic diseases in 414 families with hereditary mental retardation in Iran

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¹GRC, Tehran, Islamic Republic of Iran, ²Pediatric Department, Charité University Hospital, Berlin, Germany, ³Max Planck Institute for Molecular Genetics, Berlin, Germany, ⁴Max Planck Institute for Molecular Genetics, Germany, ⁵Pediatric Department, Charité University Hospital, Germany. Metabolic disorders account for about 3% of hereditary disorders associated with mental retardation in developed countries. Due to the progressive nature of metabolic disorders, MR is often not apparent early in life and becomes manifest only later.

In this study we asked the question what percentage of adults with hereditary mental retardation have inborn errors of metabolism and what their prevalence is in our population.

A total of 414 families were investigated by Tandem Mass Spectrometry (MS). The blood was spotted on Guthrie cards for one proband from each family. The samples were analyzed at the Charité Hospital in Berlin. Our results revealed a total of 14 positive cases among our samples (3.4%). PKU was the most prevalent form (6 families), followed by four cases of MCAD, three cases of CPT-1 and one patient with homocystinuria. The frequency of metabolic disorders in our patient cohort was 1.5% for PKU, 1% for MCAD, 0.7% for CPT-1 and 0.25% for homocystinuria. Mutation screening is under way for the positive samples to help the families with carrier detection and prenatal diagnosis.

P13.52 Type 2 diabetes risk alleles near ADCY5, CDKAL1 and HHEX-IDE are associated with reduced birth weight

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Aims: The fetal insulin hypothesis suggests that variation in the fetal genotype influencing insulin secretion or action may predispose to both low birth weight and type 2 diabetes (T2D). We examined associations between 25 confirmed T2D risk variants and birth weight in a) individuals from the Danish Inter99 population and b) meta-analyses including own data and reported studies.

Methods: Midwife records from the Danish State Archives provided information on mother's age, parity, as well as birth weight, length at birth and prematurity of the newborn in 4,740 individuals from the population-based Inter99 study. Twenty five risk alleles showing genome-wide associations with T2D were genotyped.

Results: Birth weight was inversely associated with the ADCY5-rs11708067 T2D risk allele ($\beta=-33g [-55;-10]$, $P=0.004$) and with the CDKAL1-rs7756992 T2D risk allele ($\beta=-22g [-43;-1]$, $P=0.04$). The association for the latter locus was confirmed in a meta-analysis ($n=24,885$) ($\beta=-20g [-29;-11]$, $P=8*10^{-6}$). The HHEX-IDE variant showed no significant association among Danes ($P=0.09$); however, in a meta-analysis ($n=25,164$) the T2D risk allele was associated with lower birth weight ($\beta=-16g [-24;-8]$, $P=8*10^{-5}$). On average, individuals with high genetic risk (>25 T2D risk alleles) weighed marginally less at birth than those

with low genetic risk (<25 T2D risk alleles) ($\beta=-33g [-66;0]$, $P=0.05$) Conclusions: We report a novel association between the fetal *ADCY5* T2D risk allele and lower birth weight. In meta-analyses we confirm associations between lower birth weight and the *HHEX-IDE* and *CD-KAL1* T2D risk alleles. No strong general effect on birth weight can be ascribed the 25 T2D risk alleles.

P13.53 Influence of *Wfs1* gene deletion and chronic valproate treatment on glucose metabolism and ER stress.

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The *WFS1* gene encodes a protein expressed in the endoplasmatic reticulum (ER) membrane and its deficiency causes ER stress. ER stress plays an important role in the pathogenesis of diabetes, contributing to β -cell loss and insulin resistance. The XBP1 molecule modulates the ER stress response and it is critical for the regulation of *Wfs1*. GLUT2 protein enables passive glucose movement across cell membranes with a suggestive role in the control of insulin secretion. The aim of the study was to evaluate the impact of *Wfs1* gene deficiency and chronic valproate (cVLA) treatment on hyperglycemia in mice. The gene expression of ER stress and insulin pathway molecules in liver tissue has been analyzed. The glucose tolerance test revealed that the blood glucose levels on 30 minutes after glucose administration were significantly higher ($p=0.0049$) in the *Wfs1*^{+/+} cVLA treatment group compared to *Wfs1*^{+/+} saline group. The expression of the XBP1 splice variant was significantly higher ($p=0.041$) in the *Wfs1*^{+/+} saline group when compared to the *Wfs1*^{+/+} cVLA group. In addition, a significant higher expression ($p=0.025$) of the GLUT2 was observed in the cVLA *Wfs1*^{-/-} group when compared to the saline *Wfs1*^{-/-} group. No significant differences in expression of the *Wfs1* gene between the saline and the cVLA groups were observed. In conclusion, the chronic valproate administration increases glucose levels and induces the expression of GLUT2 in *Wfs1*^{-/-} mice without improvement of glucose tolerance. The effect of VLA in insulin signal transduction pathway needs further investigation.

P13.54 Frequent *ATP7B* gene mutations in Russian Wilson disease patients

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The *ATP7B* gene mapped to 13q14.3-q21.1 encodes copper-transporting P-type ATPase. Mutations in this gene cause Wilson disease (Hepatolenticular degeneration), - an autosomal recessive disorder of copper metabolism.

The aim of our investigation was to determine the most frequent mutations in the *ATP7B* gene among Russian patients. We researched a sample consisted of 186 Russian unrelated patients of our laboratory with clinical characteristics of Wilson disease. This sample was previously researched for c.C3207A. Then we created a system for 7 mutations (deletions and insertions) search by AFLP method and tested the sample by this system. The results of the investigation are represented in a table below.

| Genotype and allele frequencies of the mutations in <i>ATP7B</i> gene in Russian Wilson disease patients | | | | | | | | | |
|--|-------------|---------------|---------------|---------------|---------------|--------------------|--------------------|--------------------|--|
| Mutation | c. C 3207 A | c. 2532 del A | c. 3402 del C | c. 2304 ins C | c. 1770 ins T | c. 1340_1343 del 4 | c. 3649_3654 del 6 | c. 3627_3630 del 4 | |
| Number of heterozygotes | 33 | 0 | 7 | 9 | 3 | 0 | 1 | 0 | |
| Number of homozygotes | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Allele frequency (%) | 23,4 | 0 | 1,9 | 2,4 | 0,8 | 0 | 0,3 | 0 | |

P13.55 Sequencing analysis of *ATP7B* gene in patients with Wilson disease in Croatia

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Wilson disease (WD) is an autosomal recessive disorder of copper metabolism resulting from the absence or dysfunction of copper

transporting P-type ATPase (*ATP7B*). More than 400 mutations of the *ATP7B* gene have been identified to date. Here we report preliminary results of sequencing analysis of the *ATP7B* gene. We have analyzed exons 5, 8, 13 and 14 of 19 clinically diagnosed WD patients from Croatia, already screened for the most common His1069Gln mutation. It accounts for 35-45% of Wilson disease alleles in a mixed European population. Genomic DNA was used to amplify 21 exons of the *ATP7B* gene. Sequencing analysis was performed by PCR and capillary electrophoresis with BigDye Terminator v3.1 kit on AB Genetic analyzer 3130xl. Out of the total number of 19 tested patients with WD, molecular analysis has confirmed the clinical diagnosis in 6 patients (31.6%) so far. Two patients are homozygous for mutations Arg616Gln and Ala1003Thr, respectively. Three patients are compound heterozygotes for mutations Ala1003Thr and His1069Gln and one patient is compound heterozygote for mutations H1069Q and 2304delC. We also found deleterious mutation in heterozygous state in three more patients: 2304dupC (two patients) and Gly591Asp. Sequencing analysis of the *ATP7B* gene is the best method to establish the frequency of mutations in specific population so the screening test panel for most common mutations can be developed for this population.

P13.56 POPULATION STUDY OF WILSON DISEASE IN BELARUS: INCIDENCE AND CARRIER FREQUENCY

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Wilson disease (WD) is an autosomal recessive disorder caused by impairment of copper transport. More than 300 mutations in *ATP7B* gene causing WD have been identified. The incidence of WD in many populations is thought to be 1:40000 and a carrier frequency is 1:90. The molecular bases of WD in Byelorussian patients have been studied. The frequency of H1069G in Byelorussian WD patients was found to be 56%. The other identified mutant alleles were 2299insC (3,7 %), 3400delC (2,4 %) and I1102T (2,4 %). The estimated incidence of WD in Belarus was found to be 1:17000 according to the correlation of the number of WD patients revealed from 2000 to 2008 with the birth rate. This value can be underestimated. We used mutation analysis approach to determine WD frequency in Byelorussian population. The screening for the H1069G mutation has been done in 560 newborns. The total number of alleles was 1120 and 6 WD alleles was revealed. This data corresponds to allelic frequency 0,53% (95% confidence interval (CI) 0,25% - 1,16%) and carrier frequency 1 in 93. Considering that H1069G mutation accounts for 56% of all WD alleles we obtained the total WD alleles frequency and carrier frequency as 0,95% (95% CI 0,55% - 1,75%) and 1 in 52 respectively. Assuming Hardy-Weinberg equilibrium, these data could be translated into a WD incidence of 1:11000 live births. The study demonstrates the importance of mutation analysis for estimation the true incidence of the disease in population.

P13.57 Increased expression of *MT2A* gene in growth delayed and zinc deficient children

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Background: Zinc has an essential role for the activity of many enzymes involved in different metabolic pathways. Zinc deficiency is resulting with diseases of growth, maturity, immunity and nervous system. However prediction of zinc deficiency is still very difficult, due to absence of a reliable marker. Since, the binding of zinc with some proteins such as Metallothionein 2A (MT2A) are crucial for zinc homeostasis, we hypothesized that zinc-binding storage protein MT2A mRNA expression level may be a biomarker of zinc status for screening.

Aim: Among normal and growth delayed children, presence of a relationship between serum zinc concentration and MT2A gene expression level was questioned.

Methods: Zinc measurements of serum and hemolysate were performed with atomic absorption spectrometry. MT2A gene expression at mRNA level was detected by quantitative PCR in cells of peripheral blood with Taqman probe. G6PDH was used as a reference. Statistical analysis was made by using Mann-Whitney test.

Results: Serum zinc concentrations were significantly reduced in

growth delayed children compared to healthy children. Expression of MT2A gene in growth delayed children was displayed a considerable induction. Within growth delayed children group, with the increase of serum zinc concentrations expression of MT2A has closed to the level of healthy children but, in the presence of lower levels of zinc, expression of MT2A has elevated significantly over control.

Conclusion: Among growth delayed children presence of the low levels of zinc was identified and, elevated expression of MT2A gene may imply an abnormal regulation of zinc homeostasis of circulating cells.

J13.1 A novel TYMP c.1001C>T mutation in a patient with MNGIE

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Mitochondrial neurogastrointestinal encephalopathy syndrome (MNGIE) (OMIM #603041) is a rare autosomal recessive progressive multisystem disorder. MNGIE is caused by mutations in the gene encoding thymidine phosphorylase (TYMP), locus 22q13. Mitochondrial dysfunction represents multiple deletions and depletion of mtDNA and ragged-red fibers on a muscle biopsy.

We describe a case of 37 year's old, subgroup Armenian (Hamshen) woman born from healthy cousins. She had been complained about hearing loss and myasthenic syndrome for the past five years. The disease manifested in childhood with mild gastrointestinal symptoms. Today she is presented with cachexia (height - 161 cm, weight - 34 kg), mild gastrointestinal dysmotility, ptosis, ophthalmoparesis, sensorineural deafness, symmetric mild low limbs weakness, decrease tendon reflexes, distal sensory loss, primary amenorrhea.

Electromyographical assay revealed peripheral demyelinating polyneuropathy with axonal loss. Needle EMG showed denervation changes without myodystrophic pattern. The decrement was detected on rapid repetitive nerve stimulation. MRI showed severe diffuse leukoencephalopathy.

Sequence analysis of coding regions of TYMP gene revealed missense homozygous mutation in the position c.1001C>T. This transition cause to substitution leucine for arginin in the 334 a.a. position of the polypeptide chain. To our knowledge this mutation wasn't described earlier.

Myasthenic syndrome in our patient seems to be a unique clinical feature that isn't usually included in the MNGIE phenotype.

P14 Therapy for genetic disorders

P14.01 Breast Cancer Susceptibility Genes Polymorphisms and pathologic effects in high risk Iranian families

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Nearly 15% of all breast cancer cases are associated with a strong genetic predisposition and BRCA1 and BRCA2 are responsible for 30% of this genetic predisposition. More than 3000 (1600 in BRCA1 and 1800 in BRCA2) different sequence variants have been reported in these genes that many of them are disease-associated, but also included are unclassified variants and polymorphisms. We investigated mutations in BRCA genes in many high risk Iranian families and determined the genetic polymorphisms in these genes. A total of 63 breast cancer patients and 50 controls were selected from subjects who had come to Kawsar Human Genetics Research Center for other purpose. All samples were fully sequenced for BRCA1 and BRCA2 genes. Many missense substitutions in BRCA1 and BRCA2 genes were identified. The missense substitution Gly1738Glu of BRCA1 is pathogenic. Here two novel mutations are reported (Gly1140Ser in BRCA1 and Glu1391Gly in BRCA2). The missense substitutions Glu1038Pro, Gly1140Ser were found in large series of breast and ovarian cancer patients aged 30-35 years and 20% < of matched controls. Based on our preliminary results some haplotypes may have a pathogenic role in breast cancer development ,the haplotypes at the BRCA1 locus defined by alleles Leu871Pro, GLu1038Gly, Ser1613Gly, Gly1140Ser was found in 10 affected families. In addition, haplotypes of BRCA1

defined by single alleles Glu1038Gly, Ser1613Gly, Gly1140ser and simultaneously Glu1038Gly, Gly1140Ser are associated with low-penetrance predisposition to breast or ovarian cancer. Further studies are required to confirm the hypothesis that genetic polymorphisms are associated with breast cancer.

P14.02 Expression of human factor IX in Drosophila S2 cell

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Gamma-carboxylation is essential for biological activities of certain proteins, including the human coagulation factor IX (hFIX), which is a plasma glycoprotein participating in the intrinsic pathway of blood coagulation. A pro-peptide sequence in the hFIX precursor is recognized by a γ -glutamyl carboxylase, to direct the carboxylation of 12 glutamic acid residues in the N-terminal portion of the molecule. This enzyme has been characterized in two invertebrates; *Drosophila melanogaster* and cone snails in addition to vertebrates. No γ -carboxylase substrate of Drosophila origin has been identified so far. However, pro-peptide of the human FIX and prothrombin are recognizable more efficiently by the Drosophila enzyme than by that of mammals. The yield of carboxylated product for the Drosophila enzyme is about five times more than that obtained with the human enzyme.

The *Drosophila melanogaster* Schnider (S2) cells were transiently transfected with a plasmid encoding the hFIX gene under the *Drosophila* metallothionein promoter (pMT). Based on ELISA the hFIX antigen was detectable in the cultured media taken from the transfected S2 cells, indicating for the potential of the insect cells for the hFIX expression. In order to evaluate the biological activity of the hFIX expressed by S2 cells in comparison with the hFIX expressed by mammalian cells, in parallel a CMV-regulated hFIX expressing plasmid was also transiently transfected into CHO cells. The two recombinant hFIX (rhFIX) expressed by S2 and CHO cells will be examined, for their γ -carboxylation and for their coagulation activities, subsequently.

P14.03 Favorable evolution after early rehabilitation in a case with Cri du Chat syndrome

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Cri-du-chat syndrome is a relatively rare chromosomal disorder with an estimated incidence of 1 in 20000 to 50000 newborns, resulting from loss of varying lengths of the short arm of chromosome 5. We present a case report on the rehabilitation treatment of a patient with this condition, aged 18 months, who received multidisciplinary treatment and precocious stimulation. The baby was born from healthy, young parents, with a birth weight of 2250 g. At examination this patient presented crano-facial dysmorphys, transverse flexion creases, important growth and psychomotor retardation, axial hypotonia and hypotrophy, bilateral varus equine, positive Babinski sign, lively deep tendinous reflexes, hypertrophic cardiomyopathy, unclosed sagittal and lambdoid sutures. The child had a happy aspect, was able to sit and to roll, but was not able to stay in the quadruped position. Rehabilitation program started at 15 months and consisted of hydrokinetotherapy in the pool for 15 minutes each day, physiotherapy for global tonisation, re-education of motor control stages, tonisation of paravertebral, quadriceps, gluteal muscles, paraffin therapy for the legs, cervico-dorsal-lumbar and leg tonic massage. Occupational therapy, coordination exercises and speech therapy were also performed. The evolution of the child was very good, he was able to stand with assistance after the first rehabilitation period of three weeks. Improvements in management of patients with this disorder by a multidisciplinary team, with the application of early rehabilitation programs increase psychomotor development, improve autonomy and finally lead to a better social adaptation.

P14.04 Moxonidine effectively alleviates excessive noradrenaline release and cold-induced sweating in two siblings with Crisponi syndrome

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Crisponi syndrome is a rare autosomal-recessive disorder involving contractions of the facial muscles, severe hyperthermia, major feeding and respiratory difficulties, physical dysmorphisms and often sudden death in early childhood. Surviving patients can develop cold-induced sweating (CIS), which has also been reported in Cold-Induced Sweating Syndrome (CISS) type 1 and type 2. CIS has not been extensively characterized in patients with Crisponi syndrome. Our aim was to effectively alleviate this symptom and to shed light on the underlying pathogenic mechanism.

We present two Turkish siblings diagnosed to suffer from Crisponi syndrome, who developed CIS during adolescence. Both patients were proven to be homozygous carriers of the c.708-709delCCinsT (Pro-238ArgfsX6) mutation in *CRLF1*. At 19°C ambient temperature, the probands showed excessive plasma noradrenaline release and profuse sweating of the upper part of the body. Treatment with clonidine was effective in reducing sweating but was associated with excessive fatigue. Moxonidine, however, ameliorated CIS and catecholamine release with negligible side effects. We propose that in Crisponi syndrome, CIS is triggered by excess noradrenaline acting centrally on α_1 -adrenoceptors and can be successfully treated with moxonidine by reducing noradrenaline release.

P14.05 Aerobics and airway clearance techniques in children with cystic fibrosis

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Background: Physiotherapy management is a key element of care for people with cystic fibrosis. Airway clearance techniques, physical exercise and inhalation therapy are part of treatment and are associated with improved long-term outcomes.

Hypothesis: aerobic fitness is an independent predictor of survival and those with better physical fitness have better quality of life.

Method and materials: 20 children with CF from Romanian National Cystic Fibrosis Center, Timisoara, with age between 10-14 years old all randomized in 2 groups: control (clearance techniques: ACTB, PD, oscillating PEP) and study (same clearance techniques and aerobic training-3 days per week, 30 minutes per session; heart rate 75% of maximum heart rate).

Objective: was to evaluate the efficiency of aerobic exercises combined with airway clearance techniques.

The results showed an improvement in all measured values of quality of well-being (the quality of sleep, respiratory manifestations, respiratory infections, number of hospitalization, fatigue during normal activities or effort, and the participation at school activities) at the study group compare to the control group.

Conclusions: Aerobic training promoted mucociliary clearance, improved maximum exercise capacity, strength and quality of life. Exercise should be considered and encouraged as part of overall physiotherapy management in CF. From time of diagnosis physical activity should be incorporated into the daily routine.

P14.06 Enzyme replacement therapy for MPS VI --- Experience in Taiwan

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We evaluated the safety and efficacy of weekly treatment with recombinant human arylsulfatase B (rhASB) in Taiwanese MPS VI patients. Eight patients (3 male, 5 female; age 1.5-21 years) receiving weekly iv infusions of rhASB 1.0 mg/kg for at least 12 months were enrolled. We assessed biochemical and clinical responses every 3 months.

Results: After 24 months of treatment, 4 patients experienced a 259.9-m (41.8 %) improvement in 12-minute walk, a 49.6-m (16.6%) improvement at 6-minute time point of the walk, and a 50-stair (36.8%) gain in 3-minute stair climb comparing with the baselines. The time require-

ment of coin picking decreased 20 seconds (46.8%). Joint Pain and Stiffness Questionnaire scores improved by 0.66 points (38.9%) in 4 patients. Improvement in pulmonary function (FVC and FEV1) was observed in 4 patients. All 8 patients achieved a mean decrease of 75.4% in urinary GAG excretion. Episodes of hypersensitivity happened on 3 patients, but the reactions resolved in 5 months after premedication. Conclusions: rhASB improved endurance, mobility, joint function, reduced GAG, and had an acceptable safety profile for most Taiwanese patients with MPS VI. ERT for MPS VI has been endorsed by the National Health Insurance program for the treatment of MPS VI in Taiwan since February, 2006. The patients in our study were not selected on the basis of disease severity, this point is in contrast with the previous clinical trials. Long term observation is needed to determine whether ERT for all MPS VI actually improves physical endurance and QoL.

P14.07 Insulin resistance in Romanian type 1 Gaucher patients

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This study investigates insulin resistance (IR) measured by HOMA parameters and the impact of Pro12Ala polymorphism of the transcriptional factor PPAR-gamma, in Romanian Gaucher patients. Thirty-six patients, 30 receiving enzyme replacement therapy (ERT), with Imiglucerase, for 3.96+/-1.23 years (group A) and 6 untreated (group B) were analyzed. PPAR-gamma polymorphism was identified by PCR-RFLP method. Twenty-six patients presented a Pro/Pro genotype (group A1) and 4 patients were Ala carriers (group A2).

Fasting glucose and insulin levels were determined by enzymatic methods. HOMA indices - HOMA-IR, QUICKI, IRI and HOMA-B - were calculated, using standard formulas.

Prevalence of impaired fasting glucose and insulin resistance among treated patients was 10% and 6.6%, respectively. Untreated patients did not show dysglycemia.

Results are summarized in table I.

In treated patients insulin resistance was directly correlated with ERT duration (significantly) and severity score index (moderately). Weight, chitotriosidase, global and pre-ERT duration of the disease had no influence on IR.

In untreated patients, insulin resistance correlated directly, moderately with chitotriosidase. Fasting insulin correlated significantly with weight, but inversely with fasting glucose, demonstrating a normal insulin sensitivity.

Conclusion: Treated Gaucher patients showed higher insulin resistance and a relative hyperinsulinemia over untreated patients, mildly correlated with severity of the disease. When treated, presence of Ala, in position 12 of the PPAR-gamma structure, improves significantly insulin sensitivity.

Table I. Insulin resistance parameters in Romanian type 1 Gaucher patients

| No | Parameter | All patients: A+B (n=36) | | | Treated patients: A (n=30) | | |
|----|----------------------------|--|---|-------|----------------------------|----------------------|--------|
| | | Group A Treated Patients (n=30) | Group B Untreated Patients (n=6) | p | Subgroup A1 (n=26) | Subgroup A2 (n=4) | p |
| 1 | Glucose (mg/dl) | 79.96+/-16.65 | 81.00+/-16.88 | 0.894 | 81.65+/-16.85 | 69.00+/-11.34 | 0.108 |
| 2 | Insulinemia (microU/ml) | 7.92+/-3.88 | 5.25+/-2.30 | 0.043 | 8.54+/-3.80 | 3.93+/-1.11 | 0.0001 |
| 3 | HOMA-IR | 1.59+/-0.89 | 0.97+/-0.23 | 0.002 | 1.73+/-0.87 | 0.66+/-0.23 | 0.0005 |
| 4 | QUICKI | 0.37+/-0.04 | 0.38+/-0.01 | 0.131 | 0.36+/-0.03 | 0.41+/-0.02 | 0.016 |
| 5 | IRI | 2.73+/-0.28 | 2.58+/-0.12 | 0.056 | 2.77+/-0.27 | 2.41+/-0.15 | 0.006 |
| 6 | HOMA-B | 213.1+/-221.2 | 136.7+/-119.7 | 0.249 | 220.6+/-234.9 | 164.0+/-97.0 | 0.417 |

P14.08 Clinical Observation: Alendronate Improves Bone Mineral Density and Quality of Life in a Boy with Gaucher Disease

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Splenomegaly, Gaucher cells in the spleen and bone marrow are the hallmarks of all 3 clinical forms of the Gaucher Disease (GD). Almost all patients also have skeletal involvement with episodes of bone pain, focal abnormalities on plain x-ray ("Erlenmeyer flask", lytic lesions) and generalized osteopenia, etiology of which is not clear. Enzyme replacement therapy (ERT) results in rapid decrease of glucosylceramide storage, but skeletal involvement seems to be totally resistant to ERT or improves too slowly. Several reports on additional to ERT bisphosphonate treatment have been published recently (Wenstrup et al., 2004; Cox, 2008).

For 2 years we observe a boy who is now 17 years old with saposin form of GD, diagnosed by typical chitotriosidase, glucocerebrosidase activity and bone marrow infiltration. At the age of 15 he had 66% bone mineral density deficiency (BMDD), pain crisis and vertebra fractures, which were the arguments to administer Alendronate with calcium and vitamin D supplementation. ERT or substrate reduction therapy were never provided. After 6 months of Alendronate treatment BMDD was 45% and after 1 year BMDD was 23%. During the course of treatment the pains were tapered and stopped, we didn't find any vertebra fractures. There were no side effects. The course of treatment with Alendronate, calcium and vitamin D without ERT also significantly improved the quality of life (QOL): the boy has higher everyday activity and makes progress in schooling.

We consider that Alendronate treatment makes BMDD lower and improves QOL even without ERT in children with GD.

P14.09 Studying the Secretory Expression of the Human Coagulation Factor IX Using *Gaussia princeps* Luciferase and Human Coagulation Factor VII Signal Peptides in Cultured Mammalian Cells

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Use of suitable signal peptide (SP) is crucial for both expression level and secretion efficiency of a protein in heterologous expression systems. Using SPs from *Gaussia princeps* Luciferase (LUC) and Human Coagulation Factor VII (FVII), we have studied the secretory expression of the human coagulation Factor IX (hFIX) in comparison with the native hFIX-SP. Considering the key role of host-preferential codon usage, RNA secondary structure and SP processing efficiency for the synthesis/secretion efficiency; we reconstructed the coding fragments of LUC and FVII SPs and used them at the N-terminal the hFIX. The chimeric fragments were examined for the secretion of the hFIX in comparison with native hFIX-SP transiently in CHO-K1 and HEK-293T cultured cell lines, in a CMV-regulated vectors. At various post-transfection times, presence of hFIX in the cultured media and cell extract were evaluated by coagulation test and ELISA. In the case of the LUC-SP in both cell lines expression of the hFIX was dramatically reduced (<1 ng/ml). In the case of the hFVII-SP containing construct, in spite of its low expression level a relatively lower intracellular FIX was documented, which indicate a relatively higher secretion efficiency of the hFVII-SP in comparison with the hFIX-SP. The native hFIX-SP construct appeared to be the most efficient hFIX expressing one, among the examined constructs. However, considerable intracellular accumulation of the hFIX for the hFIX-SP, suggest for a need for the optimization of the hFIX native SP to achieve an efficient secretion of the hFIX in such heterologous mammalian hosts.

P14.10 Reading-through premature termination codons Identified in the *BCKDHB* gene of classical Maple Syrup Urine Disease patients

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Mutations in any of the three different genes *BCKDHA*, *BCKDHB* and *DBT* encoding for E1b and E2 catalytic components of the branched-chain α -ketoacid dehydrogenase (BCKD) complex can cause Maple Syrup Urine Disease (MSUD). The disease presents heterogeneous clinical and molecular phenotypes, which ranges from classical to the mildest variant types. Up to now, 26 out of 55 MSUD Spanish patients analyzed show a defect in the *BCKDHB* gene and a high frequency of mutations resulting in premature termination codon (PTC) (14/52 *BCKDHB* alleles) have been identified in this series.

Herein, we have tested the hypothesis that some of these PTCs changes could be functionally rescued by "in vitro" and/or "in vivo" treatment with aminoglycoside antibiotics known for their ability to suppress translational stop codon recognition. Regarding the importance of both; type and sequence context of the nonsense mutation and level of nonsense transcript available for read-through, two changes, p.R285X and p.R324X have been selected as targets for the study. The efficacy of treatment has been assessed using an "in vitro" cDNA coupled transcription/translation test and the overexpression of a c-myc reporter vector containing the complete cDNA *BCKDHB* with the selected PTCs in cell lines.

Positive responses have been obtained for both changes and antibiotics, with recoveries of full length protein using TNT of around 30% and 10% of the total protein synthesized for 0.50 μ g/mL geneticin and 10 μ g/mL gentamicin respectively.

These results highlight the possible use of read-through drugs as a therapeutic option for this inherited metabolic disorder.

P14.11 Reduction of glycosaminoglycan synthesis in mucopolysaccharidosis type I cells by using siRNA

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Mucopolysaccharidoses (MPS) are severe, inherited metabolic diseases caused by deficiencies in activities of enzymes involved in degradation of glycosaminoglycans (GAGs). Incomplete GAGs degradation leads to their lysosomal accumulation in cells of patients. Recent studies indicated that substrate reduction therapy (SRT) may be a novel therapeutic approach for lysosomal storage diseases. We had previously demonstrated that siRNA-mediated silencing of genes coding for enzymes involved in the synthesis of the linkage tetrasaccharide sequence, common to both heparan sulfate and chondroitin/dermatan sulfate, resulted in a decrease in the levels of the genes' products and reduction of GAG synthesis in mucopolysaccharidosis IIIA (MPS IIIA, Sanfilippo disease) fibroblasts.

In the present study, we have used an RNA interference-based strategy to reduce mRNA levels of two genes, whose products are involved in chondroitin/dermatan sulfate synthesis: CSGALNACT1 and CS-GALNACT2, in MPS I fibroblasts. This decrease in levels of transcripts corresponded to a decrease of chondroitin/dermatan sulfate synthesis in these fibroblasts after the treatment of the cells with siRNA. These results indicate that reduction of GAG synthesis by the use of siRNA may be considered as a potential therapy for MPS I and could constitute a novel approach for MPS treatment, which may either complement or be used as an alternative approach to enzyme replacement therapy (ERT).

P14.12 Scoliosis in patients with Prader-Willi Syndrome

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Prader-Willi syndrome (PWS) is a genetic disorder with an estimated incidence of 1 in 25,000. PWS patients suffer from various medical conditions that require the attention of many professionals. Affecting up to 80% of the PWS patients scoliosis is a major concern for the orthopedist and choosing the proper treatment for these patients is challenging job because of the other clinical manifestations of PWS that can complicate the perspective. In 2008 several Romanian universities and health institution across the country joined together in a research program aimed to bring a multidisciplinary approach to PWS and Angelman Syndrome. In this article we do a review of the orthopedically problems of 14 cases of PWS that were included in this project. Age of the patients ranged between 7 and 28 years, mean 15 years.

There were 9 female and 5 male patients. Scoliosis was diagnosed in 7 cases (50%), 2 boys and 5 girls, age 9 to 28 years. In all the 7 patients the Cobb angle was less than 30 degrees and the disease were controlled only by physical exercise and regular surveillance. Nonsurgical treatment should the primarily option for PWS patients with scoliosis. Spinal surgery has a higher rate of complications than general populations. Regular physical exercises corroborated with physiokinethotherapy besides improving the spinal curve increase the muscle tonus and help maintaining body weight, and can control the scoliosis in up to 90% of the patients.

P14.13 Pseudoexon exclusion by antisense therapy in 6-pyruvoyl-tetrahydropterin synthase deficiency

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The number of mutations identified in deep intronic sequences that activate disease-causing pseudoexon-inclusion in mRNAs is increasing. Here we report the effect of cellular antisense therapy to suppress pseudoexon activation in primary dermal fibroblasts from three independent patients with mutations in the gene encoding the 6-pyruvoyltetrahydropterin synthase (PTPS), which leads to the most common form of tetrahydrobiopterin deficiency (OMIM 261640). Patient MD130 presented an insertion of 45 nt between exon 2 and 3 (r.163_164ins45) due to an intronic deletion (g.3760_3816del57) located in the 3'splice site of an inserted antisense Alu pseudoexon. Similarly, patient MD335 had inserted the same antisense Alu sequence due to a mutation (c.164-716A>T) located in the 5'splice site of the pseudoexon. In patient MD96, a 79-nt pseudoexon between exons 1 and 2 was activated by an A>T substitution (c.84-322A>T) at the 3' end of a LINE-2 sequence (r.84-85ins79). Antisense morpholino oligonucleotides directed to the 3' or 5'splice sites of the corresponding pseudoexons were designed to block intronic insertions into the mRNA for all three patients. Twenty-four hours post transfection, mRNA was isolated and transcriptional profiling analysis was performed. The RT-PCR pattern indicated that in all three cases a dose and sequence specific recovery of normal splicing was achieved. Furthermore, PTPS enzyme activity in all three patients' fibroblasts was recovered to normal values 2-3 days posttransfection. These results represent another excellent example of pseudoexon exclusion therapy in inherited metabolic disease.

P14.14 Use of read-through drugs as a novel therapeutical approach in propionic acidemia

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 Mutation-specific therapies are a promising option for human genetic diseases. Among them, the use of aminoglycosides and drugs identified after high-throughput screens such as Ataluren (PTC124) have shown to read through nonsense mutations in several diseases. In propionic acidemia, caused by a defect of propionylCoA carboxylase (PCC) involved in the metabolism of several amino acids, odd-chain fatty acids and cholesterol, nonsense mutations constitute ~10% of the total alleles in both the PCCA and PCCB genes encoding both subunits of the PCC enzyme. Among the patients' samples available in the laboratory we have selected fibroblasts with nonsense mutations in which the stop codon is UGA, which has been shown to be most susceptible to read-through. We have confirmed the greatly decreased levels of both immunoreactive protein and mRNA, probably due to the NMD mechanism. PCCA and PCCB cDNAs have been cloned in vectors under the control of the T7 promoter to allow the synthesis in a coupled transcription-translation assay (TNT). The selected nonsense mutations have been introduced by PCR mutagenesis in the corresponding vectors. The TNT assay in the presence of ³⁵SMet-Cys confirms the synthesis of truncated proteins of the expected size. Different concentrations of gentamicin and of genetin have been assayed. Our first results indicate that the inclusion of genetin (0.1-0.25 µg/mL) in the synthesis reaction results in the production of up to 10% of full-length protein. To determine if this results in a functional recovery of the defect the corresponding patients' fibroblasts will be treated with the read-through compounds.

P14.15 Salbutamol increases SMN mRNA in spinal muscular atrophy patients (SMA): relevance for clinical trial design

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Spinal muscular atrophy (SMA) is a neuromuscular disorder caused by homozygous absence of the *SMN1* gene. Based on severity, three forms of SMA are recognized (type I-III). All patients have at least one (usually 2-4) copies of a highly homologous gene (*SMN2*) which produces insufficient levels of functional SMN protein, due to alternative splicing of exon7. Recently, we have provided evidence that *SMN2* expression can be enhanced *in vitro* by albuterol/salbutamol, a beta2-adrenergic agonist. This compound has also been shown to improve motor function of SMA patients in two different open pilot trials. In the present study, we have evaluated the *in vivo* molecular efficacy of salbutamol in SMA patients. We have recruited 12 type II-III patients who received the compound orally for six months. *SMN2* full length transcript levels have been determined in peripheral blood leukocytes by absolute real time PCR, at baseline and after 3 and 6 months of treatment. A significant and constant increase in *SMN2*-full length transcript levels was detected and the response was directly proportional to *SMN2* gene copy number. Our data strongly support salbutamol as potential candidate for SMA treatment, and suggest that *SMN2* copy number may predict the molecular response to treatment and may be useful as a randomization parameter in double blind placebo-controlled clinical trial design.

P14.16 Chemical coupling of targeting moiety on phage surface; A distinct approach for transgene delivery into eukaryotic cells

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Targeting gene carriers to specific cell/tissue is of great interest. Some approaches including displaying peptide or monoclonal antibody on phage surface have been tried yet. However, taking advantage of phage display has improved gene delivery and expression of transgene to eukaryotic cells, but its efficiency has been remained to be improved. Previous studies reported that the efficiency of phage penetrating to eukaryotic cells is directly related to the copy number of displayed moiety. On the other hand, the number of phage coat fusion-bearing proteins in each phage particle is limited because of their interference at phage particle assembly process. To increase the number of displayed moieties on each phage particle, we have addressed the problem from a distinct point of view. The lambda bacteriophage particles bearing the GFP have been utilized to couple with human holo-transferrin chemically to formulate targeted lambda phage nanobioparticles. Coupling conjugation efficiency has been evaluated by ELISA using HRP-conjugated anti human apo-transferrin. Cell penetrating efficiency of targeted nanobioparticles and nontargeted lambda particles was compared using ELISA and PCR. The GFP expression was evaluated by RT-PCR and fluorescent microscopy followed by flow-cytometry. Our results revealed more efficient penetrating and delivery of transgene into AGS cell line and highlight the potency of coupling conjugation procedure as a distinctively efficient alternative route to standard phage display system in eukaryotic cell gene delivery trials.

P14.17** siRNA-mediated reduction of MBD2 results in up regulation of γ -globin in human model cells

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The β -thalassemias are congenital anemias that are caused by mutations that reduce or abolish expression of the beta-globin gene. Excess alpha-globin precipitates in erythroid progenitor cells resulting in cell death, ineffective erythropoiesis and severe anemia. The chicken homolog to an MBD2 containing NuRD co-repressor complex (MeCPC) has previously been purified from primary erythroid cells and characterized as binding to the methylated β -globin promoter in ery-

throid cells of adult chickens in which the gene is silent [Kransdorf et al. Blood 2006; 108:2836-45]. Knockdown of MBD2 by siRNA in MEL cells stably transfected with a methylated p-globin gene construct leads to a greater than 10-fold increase in p-globin gene expression. Likewise, knockout of MBD2 results in a ~20 fold upregulation of the human gamma globin gene in adult erythroid cells of β YAC transgenic mice [Ruponi et al. PNAS 2006; 103:6617-22]. Other group addressed the role of DNA hypomethylation in the induction of -globin expression by 5-azacytidine [Mabaera et al. Blood 2008; 111:411-420]. These observations provide an impetus to investigate the role of MBD2 in -globin induction and 5-aza pathway. We investigated by northern blot and quantitative PCR assay that -globin could be induced by 5-aza in the model cells. In the second step -globin levels increased in the presence of MBD2 siRNA. Finally, as predicted, treatment of cells with 5-azacytidine in the presence of MBD2 siRNA induces only a small, nonadditive induction of -globin mRNA, signifying that DNA methylation acts primarily through MBD2 to maintain γ -globin suppression in adult erythroid cells.

P14.18** The therapeutic potential of δ globin gene in "Th3/+" mouse

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The delta globin gene produces a small amount of delta globin, being part of the HbA2 synthesis.

The delta globin chain could be a valid substitute of the beta globin chain in thalassemia and also an antisickling agent in sickle cell anemia.

Our previous work *in vitro* showed that the creation of the CACCC box consensus sequence on the delta globin gene promoter is sufficient to enhance its expression to a considerable extent.

We produced a transgenic line carrying the mini LCR (HS-HS4) and the delta gene driven by the CACCC containing delta promoter (CACCC-delta-LCR).

Here we show that the delta globin gene can be activated *in vivo* reaching high levels of expression in our transgenic mouse model.

Our results on a single copy transgenic line show an expression level of the delta gene of 30% compared to the endogenous beta major.

This level of expression could be considered curative for beta thalassemia and sickle cell disease.

After this result, we decided to intercross the homozygous transgenic line CACCC-delta-LCR with the heterozygous mouse model of beta-thalassemia (Hbb-th3/+).

Hematological analysis performed at 8 weeks after-born mice Hbb-th3/+ revealed 8.7 ± 1.2 g/dL of total hemoglobin levels versus 10.5 ± 1.3 g/dL in heterozygous CACCC-delta-LCR/Hbb-th3/+ mice, 11.6 ± 0.94 g/dL in homozygous CACCC-delta-LCR/CACCC-delta-LCR/Hbb-th3/+ and 15.48 ± 1.4 g/dL in wild type mice.

We plan to obtain the final validation of the delta globin gene as a therapeutic gene by the rescue of the thalassemic mouse model th3/th3.

J14.1 Early physical therapy for children with genetic disorders

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Aim: To focus upon the crucial moments from the first years of genetic affected children's life, when an accurate diagnosis followed by well-oriented physical therapy can modulate the prognosis, in some cases. Material and methods: We worked with a cohort of kids with genetic transmitted diseases included in long and complex therapy, including physical. The diagnostic was established in the Emergency Children Hospital L. Turcanu Timisoara (between 2006 and 2009) and the physical treatment performed in ambulatory department next to the same hospital. The children's were diagnosed with Down syndrome - 11 cases, and other different diseases as Goldenhar syndrome, arthrogryposis, neurofibromatosis, Horner Syndrome, and more than 10 children with cerebral palsy. The physical specialist identifies potential or existing problems and consults the other medical team members (surgeon, psychiatrist, genetician). Results: In all cases the quantifiable outcome was documented with individual observational papers

and in some with measurements or pictures. The specific intervention was conducted with remarkable results in Down syndrome cases, the main aspect - motor development was better correct when the cases were sent to therapy in the first month of life. Conclusions: The team work with toddlers genetic disorders and an early diagnose followed by individual plan for physical therapy can give other chance for integration in future life and low the medical costs in upcoming years of existence.

J14.2 The importance of sight in early motor development

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Background: Studies made on groups of blind children and groups of children without visual impairment in similar conditions have pointed out the fact that the medium age at which the main milestones of motor development are performed, are significant delayed. Fine and gross motor development of the blind babies are crucial in order to achieve maximum independence.

Method and materials: the longitudinal study compared the developmental data concerning 9 motor skills of 11 blind children (retinopathy of prematurity) from Special Care Center "Speranta" Timisoara with age 2 months -3 years old, to a control group of sighted children at the same age.

Objectives: to establish the age when they perform the milestones; to evaluate the motor behavior of the blind children; to advise the parents how to handle their babies.

The results the motor development of blind children was delayed in all the stages, but significant in 5 motor skills that were examined ($p < 0, 05$). This delay shows the major importance of vision in motor development and in self-care skills, but also is caused by the lack of stimulation and no/poor motor experience.

Conclusions: Early intervention of the physiotherapist for the achievement of maximum potential of the child, a safe and an adequate stimulating environment, proper handling could shorten the motor developmental delay and could improve the quality of life of those children.

P15 Laboratory and quality management

P15.01 Quantitative Study of bacterial DNA effects on aged bone DNA amplification

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Molecular human identification is one of the most important tests performed in forensic laboratories. These tests are applied for identification of human remains from natural disasters, wars, etc., but two problems may occur as a result of DNA degradation and contamination. We are planning to test probable effects of bacterial DNA on amplifying aged bone DNA. DNA was extracted from bone remains and E.Coli. Despite the large amount of DNA observed, PCR amplification for molecular identification was failed. Using different aged bone and bacterial DNA dilutions along with PCR based methods, we tried to test their positive, negative or inhibitory effects on each other. Quantification of these effects is carrying out by real time PCR. Based on our preliminary data, addition of bacterial DNA is a valid biological test for testing quality of bone DNA to enable us for the identification testing of human remains. This method will help to detect DNA and DNA contamination which are usually present in the archeological remains.

P15.02 European validation of a new molecular diagnostic test for Cystic Fibrosis

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The efficacy of a new molecular screening method for CF, the xTAG® Cystic Fibrosis 71v2 kit (Luminex 200), allowing identification of 71 *CFTR* mutations and 6 polymorphisms, was compared with a routine reference method, the INNO-LiPA from Innogenetics. The latter consisting on the combination of CFTR17+Tn Update and INNO-LiPA CFTR19 allows identification of 36 mutations and 3 polymorphisms.

For validation purposes, CF positive and negative DNA samples were tested in parallel by both methods. Initially, 30 DNA INNO-LiPA positive samples were analysed by Luminex and results were compared with those obtained by INNO-LiPA. Wild-type DNA samples (n: 60) were also compared. If a new substitution was detected, a *CFTR* exonic sequencing (BDT3.1/3130xl, Applied Biosystems) was performed. Finally, DNA samples obtained from different biological matrix (10 samples for each: whole blood, chorionic villi, amniotic fluid and blood spot from Guthrie cards) were processed. Samples were analysed in triplicate by 3 different operators at 3 different days.

For all DNA samples, the new and the reference method provided similar results for each corresponding *CFTR* mutation. The reproducibility of the new method was comparable and no significant inter-operator variability was noted.

As conclusions, the Luminex kit is a robust *CFTR* screening method allowing a twice as large *CFTR* mutation detection rate and applicable in routine lab analysis. Currently more than 300 screening tests have been performed by the new method at our lab.

P15.03 Cytochrome P450 analysis using HRMA and DHPLC

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Cytochrome P450 is a superfamily of enzymes catalyzing the oxidative metabolism of many endogenous compounds and xenobiotics. Two enzymes, CYP2C9 and CYP2C19, are primarily involved in the transformation of pharmaceuticals; together they metabolize around 20% of widely prescribed drugs. Genes CYP2C9 and CYP2C19 encoding those enzymes are highly polymorphic. Many of alleles account for a deficient metabolism, resulting in phenotypes of poor (PMs) and intermediate metabolizers (IMs). In our study we investigated the three most common alleles responsible for a decreased enzyme activity: CYP2C9*2 (p.Arg144Cys), CYP2C9*3 (p.Ile359Leu) and CYP2C19*2 (c.19154G>A in exon 5, causing aberration in splicing site) in a control population of 100 patients from Saxony and Thuringia. We tested the effectiveness and reliability of DHPLC (Denaturing High Performance Liquid Chromatography) and the more recently developed HRMA (High Resolution Melting Analysis) used for genotyping as alternative methods to automatic sequencing. Both methods turned out to be equally sensitive and precise. However, HRMA required less preparation steps and allowed a higher throughput than DHPLC. HRMA showed a great potential as a rapid genotyping technique and it helps limiting the need for sequencing. Allele frequencies estimated in our study (13 % for CYP2C9*2, 5 % for CYP2C9*3 and 19 % for CYP2C19*2) were similar to the ones determined in other Caucasian populations.

P15.04 A window on the lab: one year of diagnostic activity in the Molecular Genetics Laboratory of Ferrara - Italy

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FERRARA is a small town in Emilia Romagna (Italy), but with an important Centre for Molecular Genetics, not only at regional but also at national level and receiving international requests for molecular testing. Defining both the means and the safety of genetic testing is not only important for public health care organisation planning and evaluation but also for the intense public interest and concern about anything labelled 'genetics'.

Here we intend to give an overview of the specific pathologies/genes we analyse, the number of examinations performed per year, the techniques used, and finally the participation to National or European External Quality Assessment Schemes at least for the tests quantitatively

more represented.

We believe that to correctly perform molecular diagnosis of genetic disorders a deep cultural medical genetics background is needed. The patients referring to our Lab are sent by the Genetic Counselling Service of our Department, or by other specialised Institutes. Our Lab ensures a deep and continuous interaction with requesting doctors, in order to chose the best diagnostic flow-chart for obtaining the correct interpretation of the molecular results.

Note: Please see the online version of the abstract database (www.eshg.org/eshg2010) for the table included in this abstract.

In conclusion, we believe that only laboratories with recognised and experienced background can assure consistent and accurate testing since they are able to perform extensive, up-to date molecular diagnostics by using articulated methods, and can give accurate interpretations of data.

P15.05 How to prepare samples to molecular genetic tests?

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Molecular genetic testing is often performed as part of a routine diagnostic procedure. It is often based on taking a sample of blood. This study investigates the use of a simple DNA extraction technique for application in medical and research studies and systematically analyzes the potential impact of time lag and temperature of storage between blood drawing and DNA isolation. It compares the influence of various blood sample storage conditions on the quantity and quality of isolated DNA. A modified phenol/chloroform isolation technique was used. DNA was isolated from samples collected from 13 participants and processed in triplicate: a) after storing blood at 20°C for 3 days; b) after storing blood at 4°C for 7 days; c) after storing blood at -20°C for 28 days. The polymerase chain reaction (PCR) and DNA concentration measurements were performed to analyze the quality of isolated the DNA. The amount of DNA isolated was as low as 1.37 ng/mL after storage at 4°C and 865.0 ng/mL when stored at -20°C. This pilot study suggests that storage at -20°C for 28 days and at 20°C for 3 days allows isolation of high quality DNA. Further studies are needed to investigate the influence of long-term storage of biological specimens on DNA isolation and quality.

P15.06 European external quality assessment (EQA) for Constitutional molecular karyotyping: Experiences from the CEQA/EMQN pilot scheme.

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The Cytogenetics European Quality Assessment scheme (CEQA) and the European Molecular Genetics Quality Network (EMQN) initiated a joint pilot scheme for molecular karyotyping.

Based on a questionnaire 10 labs were selected for each method (BAC-ArrayCGH, Oligo-ArrayCGH, SNPArray). 10 µgDNA isolated from a transformed cell line derived from a patient with developmental and mental retardation was sent out. The patient was carrier of two clinical significant aberrations: a 1.7 Mb telomeric deletion at 20p and a 9.1 Mb terminal duplication at 18p11.32p11.22. A 30 year old male patient with obesity, microgenitalia, no philtrum and mental retardation was presented as case scenario.

Participating labs were asked to proceed according to their standard methods and to return their results (genotype and clinical interpretation). One lab failed to perform testing. Six labs made significant genotyping errors (21%). 24 labs (83%) failed to provide an adequate interpretation. Moreover, 7 of these labs (24%) provided no interpretation at all. Only 3 labs (10%) fulfilled the required criteria (correct genotype and major aspects of the interpretation).

From this EQA, it became clear that many labs have serious technical problems to detect even large chromosomal imbalances using array CGH. In addition, this EQA reveals a large variation in the reporting methodology of those laboratories.

The scheme demonstrated that a significant proportion of the laboratories currently offering molecular karyotyping in a clinical diagnostic context are poor performers. In addition, this EQA has set criteria for reporting which will in the future result in more uniform reporting of array results.

P15.07 Customer satisfaction survey as a tool to improve the Cystic Fibrosis External Quality Assessment scheme

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The Cystic Fibrosis Network aims to provide well organized External Quality Assessment (EQA) schemes for cystic fibrosis (CF). In order to define the needs of the EQA participants and to continuously improve the scheme, a customer satisfaction survey was developed.

All 213 laboratories that participated in the CF EQA scheme round of 2008 were invited to complete an online questionnaire, which touched upon different aspects such as user friendliness of the different online forms, clarity of instructions, suitability of sample concentration, understandability of the report and complaint handling.

Responses were collected from 32% of the laboratories, located in 22 different countries, and put into an importance-satisfaction matrix. This matrix gave the scheme provider an overview of both the relative importance of the different aspects and participants' satisfaction.

The general satisfaction about the service of the CF Network was good, demonstrated by an average of 4.33 on a scale of 1 to 5. Issues that fulfill or exceed participants' expectations include clarity of the instructions to register and accurate time schedule, as well as suitability of DNA purity and sample packages. On the other hand it was shown that the CF EQA scheme could improve with respect to the online form used to submit genotype results, reports and raw data, and the understandability of individual comments and general report.

This survey and its results provide a tool to identify shortcomings and strong points, and to prepare to fulfil the requirements of ISO 17043, the new accreditation standard for EQA providers.

P15.08 Assessment of the Fragile X PCR screen assay in retrospective genotyping

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We present our initial assessment of the novel Fragile X PCR Screen assay (Abbott Molecular). Retrospective analysis was performed in 80 samples characterized previously by Southern blot, except for the normal male controls. This evaluation was performed in 22 samples with full mutations, in 21 samples with premutations, in 3 samples with alleles localized within the "grey zone", in 2 mosaics of normal alleles with full mutations, in 3 mosaics of a premutation with a full mutation and in 29 normal cases. Altogether 43 female and 37 male samples were tested. Expansions were detected using Fragile X PCR Screen assay in all samples with pre-mutations or full mutations. The signal of trinucleotide repeat peaks was clear up to 90 CGG in all female samples with full mutations and up to 115 CGG in all male samples with full mutations. Our data provided evidence that the new assay is fast and efficacious for detecting Fragile X-related expansions. Moreover, the test is useful for exact determination of borderline alleles within the normal, grey zone and permutation ranges. Based on our experience this new assay could be used as a rapid first choice to exclude the presence of an expansion alone. The current commercial Fragile X PCR 'sizing' assay from the same vendor could be used in the second step to substantiate the exact sizing of alleles at risk. In summary, both assays can provide an integrated, Southern blot free and fast diagnostics of the Fragile X Syndrome.

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P15.09 How to survive the sequence boom? Submit your mutations and unclassified variants to databases

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The ever-increasing speed of DNA sequencing and other forms of genome analysis leads to a fast growing mountain of DNA variants of unknown clinical significance. All laboratories are facing the challenge of interpreting these correctly, and provide evidence based answers to the questions asked by clinicians. Since the majority of variants are not reported in the literature, and single centers will never collect sufficient data on a variant on their own, it will remain very difficult to interpret variants under the current system.

To characterize variants we need large numbers of well-documented

patients and their family members to get a clear picture of the genotype-phenotype relation. The only way to collect sufficient numbers is by sharing the data in databases such as DECIPHER, ECARUCA, HGMD, and Leiden Open (source) Variant Databases (LOVD), etc.

This cannot be the effort of a few enthusiastic individuals who tend to their pet gene database or chromosomal region into the small hours. Reporting all detected variants to one single database should be an integral part of the quality system of the diagnostic laboratory. Each accredited laboratory, be it public, academic or commercial, should have a small share in database curation proportional to its annual turnover. Our scientific societies, national and international, should take up the challenge to co-ordinate this effort.

Funding from health insurance cannot be expected. However, in the longer run the system finances itself, since the interpretation of sequence variants will expedited. Eventually this will help diagnostic laboratories cope with their increasing workload.

P15.10 European external quality assessment for the improvement of KRAS testing

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KRAS mutational status in tumour cells has become an important determinant for the successful application of EGFR targeting therapy in patients with colorectal cancer. To provide optimal and reliable testing for patients throughout Europe, regional KRAS External Quality Assessment (EQA) schemes in 8 different European countries were set up to evaluate the performance of KRAS testing, including the assessment of the percentage tumour cells evaluated (a critical step in the testing) and the correct identification of the mutations.

Consecutive unstained sections paraffin-embedded material from 10 invasive colorectal carcinomas with known KRAS mutation status were sent to each participating laboratory. The laboratories could use their method of testing of choice and were required to provide the results within 10 working days. In total, 61 laboratories participated in the regional schemes. From these 61 laboratories, 44 reported all 10 genotypes correctly. 9, 6 and 1 laboratories made 1, 2 and 3 genotype mistakes, respectively. A wide variety of different DNA extraction methods and KRAS mutation detection methods is being used. The estimation of % tumour cells showed a large difference among the participants.

We conclude that more standardization in estimating tumour cell percentages is required but that about 70% of laboratories correctly identified the KRAS mutational status in all cases. We expect that this EQA scheme is a useful educational tool that provides information about the performance of a laboratory compared to other laboratories, finds the source of the genotype mistake(s) and eliminates them.

P15.11 Evaluation of two commercially available Multiplex Ligation-Dependent Probe Amplification (MLPA) software programs.

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In order to maintain quality assurance and control in a diagnostic lab setting, MLPA analysis is best achieved with controlled software. Our lab chose to review two commercially available MLPA software analysis programs: GeneMarker v.1.9 (Softgenetics) and Sequence Pilot MLPA v.3.3 (JSI medical systems GmbH) using the results from a number of MLPA kits purchased from MRC-Holland. These two software programs were evaluated with respect to their ease of use, audit trail capability, network performance, accuracy of allele sizing (automated binning), ease of template modification, user manual, ability to discriminate various allele copy numbers (ie. from 0 - 4 alleles), and the ease of interpretation of methylation-specific MLPA data. Furthermore, examination of the standard deviations from 100 data sets permitted a comparison of the two algorithms used by the software. These data

sets also allowed calculation of program sensitivity and specificity using real-time PCR to classify discordant or out-of-range allele calls. The results from this study have shown that both software programs are comparable and selection of software is dependent on laboratory requirements and preferences.

P15.12 Towards better mtDNA population samples by inspecting autosomal STR markers

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Mitochondrial DNA with its features of a circular genome, strictly maternal inheritance and high copy number is an intriguing marker applied in population, medical and forensic genetics and phylogeography. Reliable data are required for all these applications. Quality control measures have been introduced to ensure highest standards in sequence data generation, validation and a posteriori inspection. A phylogenetic alignment strategy has been widely accepted as a prerequisite for data comparability and database searches, for a reconstruction of human migrations and for a correct interpretation of mtDNA mutations in medical genetics.

There is continuing effort in enhancing the number of worldwide population samples in order to contribute to a better understanding of human mtDNA variation. This often means to rely on convenience samples collected for other purposes that do not meet all quality requirements for mtDNA data sets. Here, we introduce an additional quality control means that deals with this limitation: by combining autosomal marker with mtDNA information, it helps to avoid the bias introduced by related individuals included in the same (small) sample. By STR analysis of individuals sharing their mitochondrial haplotype, pedigree construction and subsequent calculation of likelihood ratios based on the allele frequencies found in the population, closely maternally related individuals can be identified and excluded. An ideal population sample would be representative for its population: this new approach represents another contribution towards this goal.

This work was supported by the Austrian Science Fund FWF, project L397.

P15.13 EMMA, an innovative diagnostic method for simultaneous detection of point mutations and large scale rearrangements: routine screening of 1224 patients on BRCA1 and BRCA2

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EMMA (Enhanced Mismatch Mutation Analysis®, Fluigent) is a new method for mutation screening based on heteroduplex analysis by capillary electrophoresis thanks to an innovative polymer. In combination with limiting PCR conditions, point mutation and large scale rearrangement are detected in a single run. High profile specificity and reproducibility allow the interpretation of polymorphisms based on their profiles. This was checked by blind analysis of 402 patients and following sequencing of all polymorphic profiles: as expected, all polymorphic profiles showed the expected polymorphism without any other variant.

We report on the routine diagnostic use of this method for *BRCA1* and *BRCA2* screening on a series of 1224 unrelated patients. *BRCA1* and *BRCA2* were amplified in 24 multiplex PCRs (81 fragments) using a single condition. PCRs were electrophoresed with a single analytical condition on an ABI3100 and data were analysed using dedicated software (Emmalys).

Mutation detection rate was 12%, which is in line with the results we previously obtained for 3700 index cases analysed by DHPLC.

This easy-to-use method relies on i) a single condition of analysis: modelling related to melting domain is not required, ii) simultaneous detection of point mutations and large rearrangements, iii) ready-to-use optimized polymer, iv) throughput: 30 cases are screened on both *BRCA1* and *BRCA2* in one week by one technician (DNA extraction and sequencing excluded) v) low reagent costs: 3 times cheaper compared to DHPLC.

Overall EMMA demonstrates considerable simplification and cost reduction with regards to previous diagnostic methods while keeping the

same sensitivity.

P15.14** Genetic investigations in paternity cases in Portugal: a call for psychosocial and ethical recommendations

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The Paternity Testing Commission of the International Society for Forensic Genetics has published several recommendations on biostatistics, laboratory management and quality control for genetic investigations in paternity cases. In this paper we aim to expand the traditional technical and scientific requirements related to the collection, use and storage of genetic information in paternity cases by exploring the unspecified practices of informed consent and individual identification performed by experts working in Portuguese laboratories involved in paternity testing.

A qualitative and interpretative design was followed, grounded on the following sources of information: (a) interviews conducted with experts involved in genetic paternity investigations ordered by courts by laboratories located in Portugal; (b) the consent forms and the individual identification sheets used in genetic investigations of paternity cases. Official technical and scientific recommendations on standard procedures and quality control in the field of paternity testing coexist with informal and heterogeneous laboratory practices. Specific recommendations on ethics and psychosocial issues related to genetic investigations in paternity cases are needed, drawing on debates around the implications of this activity to citizens' individual rights, personal autonomy and privacy. Good practices for genetic investigations of paternity cases should also incorporate guidelines concerning the anonymization of data, the storage and content of biological samples and possible uses of the genetic information, in order to guarantee the quality and safety of the genetic databases.

P15.15 Comparison of PEP and nested PCR for analysis of single cell and low quantity DNAs

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Molecular analysis of a single cell is essential in many genetic studies such as preimplantation genetic diagnosis and forensic investigations. Whole genome amplification methods have been introduced as powerful tools for DNA analysis in a single cell. A molecular technique, named primer extension preamplification (PEP), has been introduced as a method for whole genome amplification. In other hands, nested PCR has been used for amplifying DNA from single cell and very few cell samples. Here, we are planning to compare the efficiency of these techniques. DNAs were extracted from 30 blood samples, 10 bone. Also, thirty blastomeres were obtained using a standard IVF procedure. PEP procedure was applied on DNA samples and blastomeres using a 15-base random oligonucleotide primer followed by PCR amplification using specific primers for three regions (exon1 from FVIII and a segment of beta globin, GJB2 gene). These regions were also amplified by nested PCR in two steps. Briefly, for PEP-PCR analysis, 147 of 450 samples (32.67%) for FVIII gene, 122 of 450 samples (27.11%) for beta globin gene and 137 of 450 samples (30.44%) for GJB2 gene were determined. However, for nested PCR, 290 of 450 samples (65.56%), 302 of 450 (67.11%) and 295 of 450 (58.89%) were determined for FVIII, beta globin and GJB2 genes. Our preliminary data showed that good amplification efficiencies are provided when performing nested PCR. Thus, the nested PCR has a higher efficiency than PEP-PCR for single cells. Our data highlights the application of nested PCR for diagnostic purposes such as PGD.

P15.16 A standardized framework for the validation and verification of diagnostic molecular genetic tests.

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The validation and verification of laboratory methods and procedures prior to their use in clinical testing is an essential component of providing a safe and useful service to clinicians and patients.

After test design and development are complete, it is necessary to determine whether the performance of the test, in terms of **accuracy**, meets the required diagnostic standards. Whether this is achieved by performing **analytical validation** or **verification** depends on the existence of a suitable **performance specification** that details the ex-

pected accuracy of the test under given conditions. The validation or verification of methods are formal requirements for the accreditation of laboratories. Although the general requirements are clearly stated in the standards, very little guidance is available about the specific requirements and concepts in molecular genetics.

To address these shortcomings EuroGentest created a working group comprising clinical scientists and experts on quality assurance and statistics. Through a series of meetings literature review and consultation we have produced a guidance document outlining the principles of validation and verification in the context of molecular genetics. We describe implementation processes, key components, types of tests and suggest some relevant statistical approaches that can be used by individual laboratories to ensure that tests are performed to defined standards. As a practical tool we have also developed a standard *pro forma* that can be used as a plan to guide validation or verification procedures, act as a checklist to ensure all points are adequately covered and provide a framework for systematic documentation.

Abstracts of EMPAG Plenary Sessions

EPL1.1 Adapting to the new genetic status after predictive testing for Huntington's disease - the experiences of non-carriers

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Little is known about the personal experiences of receiving a negative test result after predictive testing for Huntington's Disease.

The aim of this study was to explore how the test results have influenced the non-carriers' lives, seen in a long term perspective (> 5 years).

Method: Semi-structured interviews were carried out with 21 non-carriers, tested for the HD mutation 6-12 years ago. The interviews were audio-taped and analysed with a qualitative content analysis method. Results: A broad variety of reactions to the new genetic status were revealed. The informants witnessed both positive and negative reactions. Everybody experienced a great **Relief of anxiety**, but for some it was accompanied by **Anxiety of receiving a second chance** in life. It was stressful living up to their expectations of doing something important and worthy this extraordinary lottery win. Others experienced a feeling of **Emptiness** immediately after receiving the test results. A couple of informants experienced severe problems with adapting to the test results which they described as **A need of a new identity**. A common negative reaction of the test results was **Feelings of guilt towards untested siblings or siblings affected by HD**. Two informants made major positive changes directly related to the test results: one completely changed lifestyle from being a criminal to becoming a serious student and another quit smoking on the day the test results were given.

Conclusion: This study shows the importance of long term follow-up after predictive testing to support non-carriers to adjust to a new genetic status.

EPL1.2 Early experiences with a genetic disorder: consequences in later life

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Persons who are at 50% risk for a late onset genetic disorder with high clinical severity may have experienced negative life events in childhood due to the disease process of a parent. This may have influenced their attachment process, possibly resulting in higher-than-average levels of attachment insecurity in adulthood.

Using Rolland's Psychosocial Typology of Genomic Disorders, we compare attachment style and emotion regulation in persons at risk for a fully penetrant, unpreventable and incurable neurodegenerative disorder (Huntington's Disease, CADASIL, or HCHWA-D) and in persons at risk for Hereditary Breast and Ovarian Cancer (HBOC), which is partially penetrant and for which there are preventive or treatment options. Participants are persons who apply for predictive testing (n=168), and their partners (n=96).

In our study, we find more insecure attachment in persons at risk than in partners. Persons at risk for a neurodegenerative disorder have more attachment anxiety than persons at risk for HBOC. We find insecure attachment to be significantly associated with less adequate patterns of emotion regulation and less psychological well being, which may have important consequences during the period of testing and dealing with test results.

We present our data along with clinical experiences in a predictive testing program, to show how attachment may be used to adapt psychological counseling to the specific needs of persons who apply for testing for a disorder that has influenced their lives to a considerable extent.

EPL1.3 Living with Huntington's disease from the partner's perspective

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Introduction: Huntington's disease (HD) is an incurable, autosomal dominant, late onset neurodegenerative disorder. Although the impact of the disease on medical, procreative and psychosocial issues in HD patients has been studied extensively over the last years, little data on the consequences for the close relatives, in particular the partners, is available.

Materials and Methods: A qualitative study using thematic analysis was set up to identify the psychosocial issues experienced by healthy partners of HD patients, the daily challenges with which they are dealing and their experiences as caregivers. Semi-structured interviews were conducted in 12 partners and following topics were questioned: 1/ What disease-related issues are most difficult to cope with? 2/ How has your personal life changed? 3/ Are you able to cope with these changes? 4/ Do you communicate about the disease? 5/ How do you view the future?

Results: The major problem for the partners is experiencing the virtual 'loss' of their partner in life, resulting from an increasingly antisocial behavior and lack of communication by their diseased spouse. Their partner's illness affects them to the extent that they feel as if becoming ill themselves. Nevertheless, all show great loyalty and responsibility to their diseased spouse. Communicating about the disease to family or friends is felt to alleviate the burden. None of the partners are preoccupied with the future; they handle the problems on a day-to-day basis.

Conclusions: Documenting experiences of partners of HD individuals is important to guide genetic counseling and psychological support in HD families.

EPL1.4 The Challenge of Adolescent Clients: Using Predictive Testing for FAP as a Case Study for Exploring Developmentally Appropriate Care

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Predictive genetic tests are routinely offered to young people during early adolescence as long as medical benefit is conferred by the test. Nonetheless, it can be highly challenging to engage, work with and support young people through this process due to (i) their unique developmental stage of life, (ii) the frequent and simultaneous involvement of multiple family members, and (iii) the lack of training provided to most genetic health professionals in adolescent health and development. Young people sit between childhood and adulthood; old enough to have their developing autonomy respected yet too young to be treated exactly as adults. They differ from adults in their cognitive capacities, communication styles, health risks, independence and the influence of peers. For these reasons, young people require a different clinical approach from that provided to adults. This presentation draws on findings from ten in-depth interviews with young people who underwent predictive testing for familial adenomatous polyposis (four male, six female; five gene-positive, five gene-negative; aged 10-17 years at the time of their test). Using real cases and first-hand accounts from young people, the key challenges associated with adolescent clients will be highlighted. These will then be used to propose a model of best practice for adolescent care in clinical genetics, drawing on established practice wisdom in the field of adolescent medicine more broadly. The specific challenges brought to clinical genetics by adolescent clients require distinct and separate attention if developmentally appropriate care is to be provided both now and in the future.

EPL1.5 Empowerment: Development and validation of a new outcome measure for evaluating genetic counselling interventions

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The aim in this study was to develop, and perform preliminary psychometric validation, of a new Patient Reported Outcome Measure specific to genetic counselling and clinical genetics services to capture the previously identified construct of Empowerment. Findings from previous qualitative research, and the published research literature were used to develop a draft 84-item questionnaire to capture the five dimensions of the Empowerment construct suggested by qualitative research: Cognitive control, Decisional Control, Behavioural Control, Emotional Regulation and Hope. The draft questionnaire (paper and online versions) was completed by 549 members of patient support groups for genetic conditions. Responses were subjected to exploratory factor analysis, and parallel analysis was used to identify the number of factors to extract using promax rotation. Internal consistency was calculated using Cronbach's alpha. Test retest reliability was calculated using analysis of variance. Exploratory factor analysis identified a 7 dimensional solution: Hope, Perceived Personal Control, Emotional Regulation, Family Implications, Helplessness, Referral Coherence and Control/Benefit-Finding. Hierarchical factor analysis confirmed a single overarching construct, Empowerment. 24 questions were selected to form the final questionnaire, informed by (a) the size of factor loadings (b) the issues that the qualitative research suggested were most troubling for families affected by genetic conditions (c) clinical judgement. Internal consistency ($\alpha = 0.87$) and test-retest reliability (0.86) are acceptable. The Empowerment questionnaire has potential as a clinical genetics-specific Patient Reported Outcome Measure for use in evaluating genetic counselling interventions in both research and clinical contexts. The findings may also be useful for targeting genetic counselling interventions.

EPL1.6 "All is done by Allah"? Understandings of Down syndrome in Pakistan

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The psychosocial impact of a genetic condition can only be properly understood within the wider cultural context of the affected individual and their family. This study used Q-methodology to characterise understandings of Down syndrome in Pakistan in a sample of health professionals and parents of children with the condition. Fifty statements originally developed for a UK study were translated into Urdu and Q-sorted by 61 participants. Statements reflected different attitudes towards Down syndrome in terms of its impact on the individual, their family and wider society. Using factor analytic techniques three independent accounts of the condition were identified and qualitative data collected during the Q-sorting exercise was used in their interpretation. For two accounts, conceptualisations of the 'will of God' were central to an understanding of the existence of people with Down syndrome. However, perceptions about the value and quality of life of the individual differed significantly between these accounts as did views about the impact on the immediate and extended family. The third account privileged a more 'natural scientific' view of Down syndrome as a genetic abnormality but also a belief that society can further contribute to disabling those affected. Attitudes towards prenatal testing and termination were collected and results demonstrated that a belief in the will of Allah was not always associated with a rejection of these technologies. All views were situated within the cultural and economic context of Pakistan and reflected issues associated with raising a child with a learning disability and health problems in that country.

EPL2.1 The impact of predictive gene testing for hypertrophic cardiomyopathy and long QT syndrome

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Background: Hypertrophic cardiomyopathy (HCM) and Long QT syndrome (LQTS) are inherited cardiovascular conditions for which predictive testing has become more common. The most concerning feature of these conditions is sudden death which can be prevented if those at risk are identified.

Methods: We conducted a multi-centre prospective questionnaire-based study to examine the impact of predictive testing for HCM/LQTS. Understanding of test results, risk perception, motivations for and concerns about testing and psychological impact of result disclosure were examined. Participants ($n=77$, 14-67 years old, 29 (37.6%) tested positive) were recruited from four Australian and one British site. Questionnaires were completed before testing and at 2 weeks and 3 months post-disclosure.

Results: Only one participant regretted being tested and one could not accurately recall their result. Perceptions of the likelihood of developing disease, level of worry, and the number of concerns about LQTS/HCM reported were consistent with gene test result. Younger gene positive participants were more worried about developing disease than older ones ($p=0.003$). Regression analysis adjusting for baseline scores demonstrated a higher mean anxiety ($p=0.005$) and distress ($p=0.003$) score in gene positive compared to gene negative participants at 2 weeks, but these differences were less apparent at 3 months. There was no difference in depression scores at any time point. Results did not change significantly after adjusting for potential confounders using analysis of covariance.

Conclusion: Participants were pleased to have undergone testing, understood the implications of their result and seemed to cope well psychologically.

EPL2.2 Risk factors for sudden cardiac death and follow-up in a large nationwide cohort of predictively tested hypertrophic cardiomyopathy mutation carriers

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Aims: We investigated the presence of a clinical diagnosis of hypertrophic cardiomyopathy (HCM), risk factors for sudden cardiac death (SCD), and cardiac events during follow-up in all known Dutch predictively tested asymptomatic carriers of a sarcomeric gene mutation.

Methods and results: In total 136 (30%) of 447 mutation carriers were diagnosed with HCM at one or more cardiological evaluation(s). Kaplan-Meier curves suggested slower progression to manifest HCM in carriers <40 years. Male gender (hazard ratio (HR) 1.69 [95%-confidence interval 1.20-2.37]) and age (HR per year 1.02 [95%-confidence interval 1.01-1.03]) were independent predictors for manifest disease. Thirty-three percent of carriers, with and without manifest disease, had risk factor(s) for SCD. During an average follow-up of 3.5 ± 1.7 years two carriers, both with manifest disease, died suddenly (0.13%/person-year). A high risk status for SCD (≥ 2 risk factors and manifest HCM) was present in 16 carriers during follow-up (1.1%/person-year). Age was a significant predictor for a high risk status for SCD (HR per year 1.03 [95%-confidence interval 1.00-1.06]).

Conclusions: Thirty percent of carriers had or developed manifest HCM after predictive DNA testing. Older age and male gender were independent predictors for manifest disease. Risk factors for SCD were frequently present. The low SCD rate during follow-up disallows prognostic evaluation of risk factors for SCD. Our data suggest that SCD risk is low, that risk stratification for SCD can be omitted in carriers without manifest disease and that frequency of cardiological evaluations can be decreased in carriers <40 years as long as hypertrophy is absent.

EPL2.3 Adolescents with implantable cardioverter defibrillators: a patient and parent perspective

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An implantable cardioverter defibrillator (ICD) is a device used in the treatment of individuals with life-threatening cardiac conditions. These include genetic disorders such as long QT syndrome, hypertrophic cardiomyopathy and Brugada syndrome, which are associated with sudden cardiac death. The ICD detects abnormal heart rhythms and delivers a shock to the heart to restore it to a normal rhythm. Adult ICD patients consistently report elevated levels of anxiety and depression, as well as negative lifestyle changes associated with the device. Compared to older ICD recipients, young patients face decades of life with the device and the long term impact and implications are important to consider.

This presentation draws on findings from qualitative interviews to explore the experience of living with an ICD as an adolescent. Six adolescents and six of their parents participated; three of the six participants had received an ICD as part of treatment for a genetic cardiac condition. Eight key themes emerged: (1) Restrictions, (2) Adjusting to Life, (3) Professional Communication, (4) Benefits, (5) Ongoing Challenges, (6) ICD Shocks, (7) Not Being Normal, and (8) Holding Back. Some themes reflect experiences previously reported in the literature such as the restrictions adolescents face, and the ICD shock experience. New findings have also emerged relating to communication between health professionals, patients and parents, and the limitations adolescents impose on themselves post-ICD implantation. These findings have important implications for medical management, and may inform genetic counselling practice to ensure adolescents with ICDs and their families are managed sensitively and effectively.

EPL2.4 Congenital heart defects and heredity: what do adult patients know and want to know?

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Most patients with congenital heart defects (CHD) survive to reproductive age. Inheritance and transmission of CHD to offspring are important issues in this population. We evaluated patients' knowledge and concerns on the heredity of their CHD and the information patients received from health care providers. A questionnaire was sent to 490 adult patients with (non-syndromic) CHD aged 20 to 45 years in one university hospital. Until now, 328 patients (67%) completed the questionnaire. Interim analysis of 145 patients (49% male, mean age 31.7 ± 7.5 years) showed that only 28% of patients recalled to have received information on heredity of their CHD from their cardiologist. Eleven percent of patients consulted a clinical geneticist. Patients estimated the recurrence risk for CHD in their (future) offspring correctly in 48%, too low in 8% and too high in 44%. Additional information on the heredity of their CHD was desired by 47% of patients. Worries about transmitting CHD to offspring were reported by 49%. Patients who estimated the recurrence risk too high were more often worried than patients who estimated the recurrence risk correctly ($P = 0.03$), and more often desired additional information ($P = 0.03$). Other predictors for inadequate knowledge, concerns and desire for additional information will be discussed. We conclude that a subset of adult patients with CHD lack adequate knowledge on heredity and recurrence risk of their CHD and a large proportion of patients desire additional information. This study shows that better patient education on heredity of CHD is needed.

EPL2.5 Family letters are an effective way to inform relatives about inherited cardiac disease

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Purpose: Increasing numbers of patients are being referred to cardio-genetics outpatient clinics with potentially inherited cardiomyopathies (CM) and arrhythmias (ARR). To inform family members at risk, we ask index-patients to distribute "family-letters" containing information on risks, possibility of (genetic) screening and preventive options to close relatives. The goal of this study is to assess the response to these letters in terms of actual referral to a cardiologist and/or clinical geneticist.

Methods: Fifty-six index-patients had been asked to distribute 249 family-letters: 85 in the ARR group and 164 in the CM group. We analyzed:

(1) Whether these family-letters had reached relatives and whether relatives had been referred to a cardiologist/clinical geneticist. Therefore a questionnaire was sent to 52 index-patients.

(2) The numbers of family members that were referred to the clinical geneticist and/or cardiologist by studying our files at the genetics department.

Results: (1) Fifty percent of index-patients responded to the questionnaire; 23/26 (88%) had passed the letters on to their relatives. In 19/23 index-patients ≥1 family-member had been screened. (2) Within a mean follow-up period of 2 years the number of relatives actually referred differed significantly ($P<0.01$) between the ARR (80%) and CM group (45.1%).

Conclusions: A high percentage of family-members of index-patients with a potentially inherited cardiac disease undergo screening, particularly in the ARR group, suggesting a higher anxiety in this last group. This study shows that distribution of "family-letters" is an effective means to inform and motivate relatives to undergo screening for high risk inherited cardiac disease.

EPL2.6 Prenatal and preimplantation diagnoses in Marfan syndrome: the point of view of French patients and geneticists

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EPL3.1 Desirability of early identification of Duchenne Muscular Dystrophy (DMD): parents' experiences of the period prior to diagnosis

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Duchenne Muscular Dystrophy (DMD), X-linked recessively inherited, is the most common progressive muscular disorder in children. Early diagnosis could offer opportunities for timely initiation of treatment possibilities, genetic counselling, and prevent a long diagnostic quest. Despite the availability of a test, DMD is not included in the newborn screening programs. Ethical concerns, e.g. parents would not be able to enjoy the first carefree years of their child before learning that the child is affected with DMD, are considered strong arguments for not considering DMD screening. However, this has never been properly assessed.

Aim: This study aimed to explore the way in which parents experienced the period from their child's birth to the time the diagnosis was made. **Method:** A qualitative face-to-face semi-structured interview was held with parents of sons affected with DMD.

Results: 8 parent-couples, invited by the Dutch Duchenne Parent project, participated. They reported minor worries starting shortly after birth, increasing over time. All parents wished they had known the diagnosis earlier, preferably before the child was two years of age, for they regretted the way they had treated their child during the period in which they were unaware of the disorder in their child.

Conclusion: A true carefree period seemingly does not exist. Early diagnosis would have enabled parents to treat their child in the best possible way, adjusted to their child's condition ('good parenting'). This emerging need for 'good parenting', parents expressed, by far outweighed the possibility of enjoying a carefree period in the child.

EPL3.2 New non-invasive prenatal genetic technologies: Public understandings and concerns

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'Non-invasive prenatal diagnosis' (NIPD) is a group of novel technologies which has the potential to allow earlier and safer genetic diagnosis of the fetus in the womb from a sample of maternal blood. At a clinical level, this represents an advantageous risk reduction in comparison with existing invasive prenatal diagnostic techniques, such as amniocentesis or chorionic villus sampling. Existing academic work has focused primarily on the bioethics of NIPD, particularly over fears of exacerbating routinization and the problem of obtaining informed consent. However, little if any research, has considered what the public themselves think of the introduction of NIPD technologies. This study aimed to identify shared 'lay discourses' on NIPD amongst a sample of 71 UK participants using Q-methodology, a form of factor analysis. Participants were asked to read a short description of NIPD, and then sort 70 statements on the topic on whether they agreed or disagreed with them. A by-person factor analysis resulted in a seven factor model, with each factor representing a different lay discourse. Factors differed on several dimensions; the framing of termination decisions, the right to know/not know and perceived societal impact of NIPD. A consensus emerged over fears of commercial testing misuse and over 'trivial' testing, with a desire for ongoing regulation by health professionals. However, discourses were strongly polarized. In conclusion, amongst this sample in the UK, there was no one public understanding of NIPD. This raises challenging questions about how public attitudes towards NIPD should be incorporated into policy decisions.

EPL3.3 Attitudes and intentions to undergo invasive prenatal testing: The moderating role of ambivalence

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Objective: Attitudes strongly predict prenatal screening decisions, with ambivalence as a moderator. This study examined ambivalence as a moderator of the relationship between attitudes and uptake of invasive prenatal testing. **Methods:** Eighty-four pregnant women referred for prenatal genetic counseling completed measures of attitudes, ambivalence and intentions to undergo invasive prenatal testing. Telephone calls were made to participants four weeks later to learn of their test decision. **Results:** Attitudes were a strong predictor of both intentions and test uptake. The correlations between attitudes and intentions and between attitudes and uptake were greater in women with lower lev-

els of ambivalence ($r=0.76$ and $r=0.49$, respectively) than in women with higher levels of ambivalence ($r=0.45$ and $r=0.03$, respectively). The difference between the correlation coefficients for each, intentions and uptake, was significant ($p=0.0294$ and $p=0.0192$, respectively). **Conclusion:** These findings suggest that higher ambivalence modifies the relationship between attitudes and test uptake in invasive prenatal testing decisions. **Practice Implications:** Reducing ambivalence among women facing decisions about prenatal testing may be a useful counseling intervention to enhance informed choices.

EPL3.4 Reproductive decisions of couples with an increased risk of having a child with retinoblastoma, a cross-sectional survey

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Aim: To investigate reproductive decisions of individuals at risk for a child with retinoblastoma (Rb), and examine factors influencing these decisions.

Methods: Cross-sectional questionnaire survey, 1-10 years post-ge- netic counseling of individuals with an increased risk of a child with Rb who visited the National Retinoblastoma Treatment Center in the Neth- erlands. Risk for having a child with Rb ranged from <1% to 50%.

Results: The response rate was 69% (81/118). Rb influenced past reproductive decisions in 25 respondents, by refraining from having (more) children ($n=17$), undergoing sterilization ($n=3$) or using prenatal diagnosis ($n=5$). Sixteen respondents had accepted their increased risk and had more children, although five of them indicated they have had doubts about the decisions made. Of the 40 respondents who did not have (more) children after being aware of their increased risk, 25 did not have a future desire to have children for reasons other than retinoblastoma (e.g. family completed, gynaecological problems). Of the 15 remaining respondents, who indicated to have a future child wish, 11 indicated intentions to use prenatal diagnosis, preimplantation genetic diagnosis or adoption, because of their increased risk. The most important factor that influenced reproductive decision-making was per- ceived risk, more than actual risk ($p<.05$).

Conclusion: Rb has had a substantial effect on past and future repro- ductive decisions. Ongoing access to genetic counseling and support in the decisional process of couples at increased risk of having a child with Rb is warranted.

EPL3.5 Longitudinal evaluation 7 years after termination of pregnancy (TOP) on the context of prenatal diagnosis

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TOP, when a fetal problem is diagnosed, frequently generates consider- able emotional problems, however the lack of research concerning long term consequences is evident. Our goal is focused on obtaining information about the long-term responses of perinatal grief, trauma and depression and its evolution, after TOP on the context of an ad- verse prenatal diagnosis.

On the present evaluation, the following instruments were applied: semi-structured interview, Impact of Event-Revised Scale (IES-R), Perinatal Grief Scale (PGS) and Beck Depression Inventory (BDI). Our sample consists of 28 women with history of TOP seven years ago, which consented to participate and have been evaluated also on previous moments, 15 days (BDI) and six months after (BDI and PGS).

On the evaluation seven years after TOP we observe 21.4% of women with depression ($BDI>12$) and 42.9% with high levels of traumatic stress (IES-R>35). Comparing the previous evaluations of depression, we found that there is a considerable decrease between first and sec- ond evaluation ($p<0.01$), however there is no decrease on seventh year. Considering perinatal grief symptoms there is no significant de- crease after the sixth month. Attrition rates were analyzed and the lon- gitudinal bias controlled.

The results show a high degree of traumatic symptoms, even seven

years after TOP, describing the termination of pregnancy as a particularly intense life event, which repercussions arise continuously, emphasizing the importance of a meaningful support and regular monitoring. Understanding of these symptoms should be considered key element in future approaches of the genetic counselling on the peculiar context of the TOP.

EPL3.6 Exploring health professionals' views on Perinatal Hospice care: a qualitative study.

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Pregnant women receiving a diagnosis of a 'lethal fetal abnormality' may choose to either terminate their pregnancy, or continue the pregnancy in the knowledge that their baby is likely to die before or shortly after birth. In some countries perinatal hospice care is offered in these circumstances. It comprises provision of medical and psychosocial care within a multidisciplinary team. This caring environment allows families to engage with the ongoing pregnancy and value whatever time they have with their baby.

This presentation explores the views and experiences of key informant health professionals in Victoria, Australia involved in caring for these women about a) the current practice of care for women who choose to continue a pregnancy diagnosed with a 'lethal fetal abnormality', and b) the acceptability of perinatal hospice care. Eight semi-structured interviews were analysed using a qualitative approach allowing a number of themes to emerge.

Findings revealed that:

-The term 'lethal fetal abnormality' was viewed by this cohort as an inaccurate and inadequate term for these frequently complex situations.

-Current practice in Victoria is variable, commonly referred to as 'ad hoc', with some components of care needing improvement.

-All participants were supportive of perinatal hospice care and made suggestions about other factors to consider.

-A number of professional issues emerged including the need for education and support for professionals working in this area of practice.

These findings have practice implications for health professionals and women who choose to continue a pregnancy diagnosed with a 'lethal fetal abnormality'.

EPL4.1 What information do cancer genetic counselees prioritize?

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Purpose: The study objective was to explore counselees' expectations and the preferred issues to be discussed prior to the first genetic counseling due to a personal and/or familial history of breast or colorectal cancer.

Method: 110 counselees attending the cancer genetic counselling in 2009 constituted the study sample. The data was collected using Q-methodology and the QUOTE-GENE[®] questionnaire. Counselees ranked 30 items, based on the order of importance to them. The responses were forced onto a quasi-normal distribution, asking the participants to identify the 3 most important, 3 least important, 7 very important, 7 next to least important and 10 items that were neither important nor unimportant.

Results: The counselees expected the counsellor to be skilled, provide them with clear explanations and risk estimations. The most important issues for counselees were to receive regular clinical examinations and information about symptoms that are important to be attentive to and seek further medical follow-up for. To receive general information on genetics, reassurance, discussing emotional issues, and receiving supportive calls or receiving support in communication with relatives was regarded as the least important topics.

Conclusion: Unlike previously assumed, emotional and communicative difficulties relating to cancer genetic information, was not among the most important issues for the counselees. The counselee's highest priority appeared to be the practical aspects of the cancer susceptibility, to receive counselling including medical and risk information and

suggestions how to reduce their risk. Receiving emotional support or help in communicating the information to at-risk relatives was regarded as least important.

EPL4.2 Managing anxiety in individuals undergoing cancer genetic risk assessment: a self-help coping intervention can reduce psychological distress and avoidance of genetic risk information.

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Undergoing cancer genetic risk assessment and testing can cause psychological distress in vulnerable individuals. We have developed a self-help written coping intervention, based upon psychological theory, which teaches patients strategies to control intrusive worries whilst waiting for cancer genetic information.

The impact of the intervention was evaluated through a national randomised controlled trial. Referrals into the Cancer Genetics Service for Wales over a 12 month period were randomised into intervention or control condition and completed questionnaires upon referral (Q1), four weeks later (Q2) and one month post-risk notification (Q3). 590 participants completed Q1, 429 completed Q2, and 280 completed Q3 with an equitable response rate across trial groups. Key psychological outcomes included psychological distress, intrusive thoughts, and negative mood.

The intervention significantly reduced intrusive thoughts in those reporting moderate worries ($p<0.05$) whilst having no adverse longer-term impact in the sample as a whole. Participants in the control group (but not the intervention group) who dropped out of the study reported significantly higher levels of avoidance (mean=11.49) than those who remained in the study (mean=8.99, $p=0.05$), and had significantly higher levels of negative affect (mean=19.94) than those who remained in the study (mean=16.91, $p=.004$).

The intervention offers an effective means of controlling worries in large patient populations undergoing genetic assessment or other cancer screening programmes. The intervention may also reduce the number of patients likely to drop out of such programmes due to high levels of avoidance or negative affect.

EPL4.3 Quality of life after breast cancer: information provision regarding DIEP flap and implant breast reconstruction

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Purpose: Breast reconstruction (BR) after (prophylactic) mastectomy has shown to improve quality of life in patients who deal with breast cancer. Exploring aspects of information provision regarding BR can help improve counseling concerning the decision-making process of BR. This might make BR more accessible and the decision for a specific type of BR easier. We explored the informational and psychological characteristics of patients who either undergo DIEP flap BR (DBR) or implant BR (IBR).

Methods: This study was part of a multi-centred prospective follow-up study ($N \approx 200$). We included women who were about to undergo DBR or IBR. Preoperatively, women filled in psychological questionnaires and a study-specific questionnaire regarding information provision concerning BR. Analyses were aimed at the specific psychological characteristics that we associated with actively seeking information regarding BR.

Results: Preliminary results show significant differences between the reconstruction groups in the use of informational sources and the extent to which one makes decisions autonomously. An active coping strategy and more autonomy are associated with choosing for DBR. The physician/plastic surgeon appeared to play an important role in the decision-making process of BR.

Conclusion: To our knowledge, this is the first study exploring informational and psychological characteristics of patients who either undergo DBR or IBR. Differences found between the two groups in aspects concerning information provision, suggest information provision regarding BR is not well standardized. This is an important implication for patient counselling which can be improved to make reconstruction options clear and accessible for all patients considering BR.

EPL4.4 Acceptance, experiences and information preferences of young women newly diagnosed with breast cancer regarding treatment-focused genetic testing

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Background. An increasing number of women newly diagnosed with breast cancer and with a family history of breast/ovarian cancer or with other features indicative of a high mutation carrier risk are being offered genetic testing to guide their treatment (treatment-focused genetic testing - TFGT). This qualitative study aimed to identify young women's attitudes; prior experiences if any; and information preferences regarding TFGT.

Methods. Women (N=26) with breast cancer (<50 yrs) who had either (i) previously had TFGT (N=14) or (ii) had a recent breast cancer diagnosis and were asked about their hypothetical views of TFGT (N=12) participated in semi-structured interviews. The interviews were transcribed verbatim and coded using NVivo software.

Results: TFGT was highly acceptable. Most women wanted to be informed about it at or around the time of their cancer diagnosis. The primary motivation for TFGT was to obtain information to inform appropriate surgical decisions. Assisting family with risk information was another significant motivation, while a small number also identified decision-making about reproduction and breast reconstruction. Half of the women preferred to receive the information from a genetics specialist, genetic counsellor or their breast care nurse, while half preferred to receive information from their surgeon or medical oncologist. The preferred format for the educational materials was brief written information.

Conclusion: TFGT is highly acceptable to many women, and they want to be informed about the testing around the time of their breast cancer diagnosis. We are currently in the process of developing and pilot-testing a brief pamphlet on TFGT.

EPL4.5 Genetic counseling between breast cancer diagnosis and treatment: Psychosocial burden and clinical consequences

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Background: In 15% of high-risk families, a BRCA1 or BRCA2 gene mutation can be found. Female carriers with breast cancer have an increased risk of new primary tumours and may opt for preventive surgery. Our aim was to evaluate the psychological impact and treatment consequences of rapid genetic counseling and testing (RGCT) offered in the period between breast cancer diagnosis and initial surgical treatment.

Methods: Female breast cancer patients, who had received RGCT at the Family Cancer Clinic of the Netherlands Cancer Institute between January 2006 and November 2008, were invited to complete a questionnaire in 2009.

Results: Of the 31 eligible patients, 27 completed the self-report questionnaire. In total 26 patients had rapid counseling and DNA-testing, one had rapid counseling only. Ten BRCA mutations were found. Almost all mutation-carriers (n=9) opted for a bilateral mastectomy, compared with 44% of the other patients. Despite frequent worries about cancer recurrence being reported by 19% of the women, daily functioning was not impaired by these worries. Ten patients received psychosocial counseling at the time of RGCT; 8 reported that this was (very) useful. More than 90% of patients were (very) satisfied with the timing of RGCT and with the decisional process.

Conclusions: This small retrospective study suggests that RGCT in high-risk breast cancer patients may influence surgical treatment, without causing long-term distress. The offer of psychosocial counseling

at the time of RGCT was valued. Currently, a large, prospective randomized clinical trial is being conducted to investigate these issues further.

EPL4.6 Psychological impact of MSI testing shortly after CRC diagnosis

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Introduction: The aim of the present study is to determine psychological distress and effects on family communication in recently diagnosed patients with CRC after MSI testing.

Method: Newly diagnosed patients (n=400) with CRC from 33 Dutch hospitals whose CRC had been tested for MSI were identified by the researcher and invited to participate by their surgeons. Patients received questionnaires immediately after MSI-test disclosure (T1) and 6 months later (T2).

Results: Response rates of patients with high (MSI+) and with low (MSI-) risk for Lynch syndrome were 30% (n=23/77) and 18% (n=57/323) respectively. At T1, mean levels of cancer specific distress (IES-cancer) of MSI+ patients and MSI- patients were high (25.1, SD 9.4 and 26.2, SD 4.7, respectively) but not statistically significant different. Levels of cancer specific distress reached a post traumatic stress disorder level (IES >26) in 49% of this newly diagnosed patient group, consisting of 9 MSI+ patients and 30 MSI- patients (P=0.16). At T1, mean levels of general psychological distress (SCL-90) of MSI+ patients and MSI- patients were 137.0 (SD 43.9) and 128.7 (SD 36.2) respectively (P=0.4). MSI+ patients had statistically significant less family communication compared to MSI- patients, 12.8 (SD 5.5) and 15.9 (SD 4.5) respectively (P=0.01). Results of T2 are expected in May 2010.

Conclusion: Newly diagnosed patients at high risk for hereditary CRC report similar levels of psychological distress, but had less open family communication as compared to patients who are at low risk for hereditary CRC.

EPL6.1 Communicating BRCA1/2 genetic test results within families: implications for genetic counselling

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Background: Communication of genetic risk information is central to clinical genetics. In the UK patients are encouraged to disseminate risk information throughout their families, but the processes involved in doing this and subsequent impact upon family relationships are not well understood. This study explored the flow of BRCA1/2 test information through families, factors that influenced communication, and the process and outcomes of communication within the family.

Method: Results consultations with 10 women affected with breast/ovarian cancer receiving positive BRCA1/2 genetic test results were audio-recorded. Semi-structured interviews were later conducted to explore the women's understanding of their results and experiences of communicating this information to relatives. For each family, similar interviews were then conducted with two relatives with whom test results had been shared. Data were analysed using Interpretative Phenomenological Analysis.

Findings: Analyses indicated that not all information was accurately communicated or understood by relatives, and not all relatives were informed of results. Three themes emerged: responsibility to tell; emotional and developmental readiness and communicating in the context of the existing family culture.

Discussion: These findings highlight the importance for clinicians of communicating genetic information in the context of the family's existing communication patterns and relationships; of acknowledging the influence of feelings of responsibility and obligation within families and of supporting patients in considering who will take responsibility for communicating test results to relatives, and what will happen if their normal channels of communication are unavailable.

EPL6.2 Psychological distress in women at risk for hereditary breast cancer: the role of family communication and perceived social support

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Background: Hereditary breast cancer has a profound impact on individual family members and on their mutual communication and interactions. The way at-risk women cope with the threat of hereditary breast cancer may depend on the quality of family communication about hereditary breast cancer and on the perceived social support from family and friends.

Objective: To examine the associations of family communication and social support with long-term psychological distress in a group of women at risk for hereditary breast cancer.

Methods: The study cohort consisted of 222 women at risk for hereditary breast cancer, 4-9 years after they participated in studies on the psychological consequences of either regular breast surveillance or prophylactic surgery. Two months before a surveillance appointment at the clinic general and breast cancer specific distress, hereditary cancer related family communication, perceived social support and demographics were assessed.

Results: We found that open communication about hereditary cancer within the family was associated with less general and breast cancer specific distress. In addition, perceived support from family and friends was indirectly associated with less general and breast cancer specific distress through open communication within the family.

Discussion: These findings show that family communication and perceived social support from friends and family are of paramount importance in the long-term adaptation to being at risk for hereditary breast cancer. Sources of support and family communication about hereditary cancer need to be addressed and explored by clinicians working with women at risk for hereditary breast cancer.

EPL6.3 Is telegenetics as effective as face-to-face consultations for hereditary breast/ovarian cancer genetic counseling?

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Purpose: Telegenetics is increasingly being used to deliver familial cancer services for hereditary breast/ovarian cancer (HBOC) to outreach areas; however there has been little research evaluating this method of service delivery. This study aimed to evaluate the effectiveness and acceptability of genetic counseling through videoconferencing. **Methods:** Three hundred women were recruited between December 2007 and December 2009. Ninety-three women seen by videoconferencing and 78 women seen face-to-face participated in the evaluation. A questionnaire was administered prior to genetic counseling, and one month post-consultation. The questionnaire measured knowledge of HBOC, expectations, satisfaction, perceived personal control, perceived clinician empathy, cancer-specific anxiety, generalized anxiety and depression. **Results:** No significant differences were found between telegenetics and face-to-face genetic counseling in terms of changes in knowledge ($p = 0.36$), patient satisfaction ($p = 0.18$), perceived empathy of the genetic clinician ($p = 0.20$), and depression ($p = 0.67$). Telegenetics performed significantly better than face-to-face counseling in meeting patients' expectations ($p < 0.001$), promoting perceived personal control ($p < 0.001$), reducing breast cancer-specific anxiety ($p < 0.001$), and in terms of perceived empathy of the genetic counselor ($p = 0.03$). By contrast, face-to-face counseling was more effective than telegenetics in terms of reducing generalized anxiety ($p = 0.007$).

Conclusion: Telegenetics performed as well as, if not better than, traditional face-to-face genetic counseling in all outcomes other than generalized anxiety. It appears to be an acceptable and effective method of delivering genetic counseling services for HBOC to outreach areas.

EPL6.4 A whisper-game perspective on the family communication of DNA-test results: A retrospective study on the communication process of BRCA1/2-test results between genetic-counsellor, proband and relatives

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Purpose - We analyzed how DNA-test result information was communicated and perceived within families.

Method - We conducted a retrospective descriptive study in 13 probands with an unclassified variant, 7 with a pathogenic mutation, 5 with an uninformative result, and in respectively 44, 14, and 12 of their relatives. We examined differences and correlations between: (a) information actually communicated by genetic-counselors, (b) probands' perception, (c) relatives' perception. The perception consisted of recollections and interpretations of both cancer-risks and heredity-likelihood.

Results - Differences and low correlations suggested few similarities between the actually communicated information, the probands' and the relatives' perception. More specifically, probands recalled the communicated information differently compared to the actually communicated information ($R=.40$), and reinterpreted this information differently ($R=.30$). The relatives' perception was best correlated with the proband's interpretation ($R=.08$), but this perception differed significantly from their proband's perception. Finally, relatives reinterpreted the information they received from their proband differently ($R=.25$), and this interpretation was only slightly related with the original message communicated by the genetic-counselor ($R=.15$). Unclassified-variant were most frequently misinterpreted by probands and relatives, and had largest differences between probands' and relatives' perceptions. The low correlations between the proband's and relatives' perception could be explained by the way in which the proband had communicated the DNA-test result, especially the amount of provided reassurance.

Discussion - Like in a children's whisper-game, many errors occur in the transmission of DNA-test result information in families. More attention is required for how probands disseminate information to relatives. Genetic-counselors may directly communicate to relatives, e.g. via letters.

EPL6.5 Taking on passing on: A grounded theory on containing cancer in BRCA carriers

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Background: Psychosocial research into the BRCA1/2 breast cancer susceptibility genes has yet to account for any long term psychosocial effects of genetic testing in gene carriers who have a personal history of HBOC.

Aim of Study: To address this deficit in the literature this study was concerned with exploring the experience and needs of this group of women over time.

Methodology: A grounded theory approach was taken using qualitative interviews ($n = 42$) and reflective diaries.

Analysis and Results: Containing cancer emerged as the basic social psychological process through which BRCA carriers with a personal history of HBOC respond to and resolve what is for them, a major concern - the passing on of a cancer gene to their offspring.

Constant comparative analysis of the data traces the development of the process through the stages (1) Hypothesising Risk, (2) Formalising Risk (3) Minimising Risk and Maximising Survival

The theory adds to the literature on coping and adaption, health psychology theories and dimensions of women's health in relation to their multiplicity of roles.

Implications for Clinical Practice: This prospective longitudinal study adds to clinical practice by contributing to our understanding of how women cope with learning their genetic status and manage its implications at a personal, familial and societal level over time. It has highlighted ongoing needs of women after they leave a cancer genetic clinic and part of the genetic counselling role would be preparing

women and other pertinent health professionals in how best to meet the needs of this group.

EPL6.6 Genetic testing for BrCa and Huntington's disease in twins: experience of 10 cases

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We present our experience with 10 families with presumed identical twins/triplets presenting to the genetic service for discussion about genetic testing for BrCa or Huntington's disease. The cases included one set of female triplets, and all but one of the twin pairs were also female. Seven cases involved presymptomatic genetic tests, the other 3 were diagnostic tests for one twin and presymptomatic for the other. For half of the cases, both twins were seen in our genetic clinic, for the other half the second twin was either seen in another genetic service or did not have a genetic consultation for a variety of reasons. In all but one case, decisions about testing were agreed between the twins/triplets. In the remaining case the presenting twin was encouraged to make contact with her estranged twin in another country, but has not proceeded to testing.

We encountered several issues relating to the process and outcome of testing, including consent, value of zygosity testing, relative preparedness for testing, coordination of result disclosure, as well as impact of result for twins and the family. We will discuss strategies which we found helpful in genetic counselling management of these cases, including using zygosity testing to increase preparedness for the test result, encouraging the initially presenting twin to consider the implications of testing for their twin and on the twin-twin relationship, and careful planning for timely progression and result giving.

EPL7.1 Descriptive and numeric estimation of risk for psychotic disorders among affected individuals and relatives: implications for clinical practice

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Background: Psychotic illnesses cumulatively affect 3% of the population. Affected individuals and their families want information about psychosis risks for other family members. Deriving accurate numeric probabilities for psychosis risk is challenging, and people have difficulty interpreting probabilistic information, thus it is tempting for clinicians to use risk descriptors rather than numbers. Relationships between families' perceptions of numeric probabilities and risk descriptors in the context of psychosis are unknown. **Purpose:** We explored numeric and descriptive estimations of psychosis risk in a cohort of individuals with psychotic disorders and unaffected first-degree relatives. **Method:** An online survey was posted on an Early Psychosis Intervention website. Respondents numerically and descriptively estimated risk for an individual to develop psychosis in two scenarios. In scenario 1, the individual had no affected family members, in scenario 2, the individual had an affected sibling. **Results:** 250 affected individuals and 268 first-degree relatives estimated numeric probabilities and attributed a descriptor in at least one scenario. Affected individuals significantly estimated higher risks than relatives. Numeric probabilities of 1%, 10%, 25% and 50% were attributed all descriptors between "very low" and "very high" by respondents. There were significant differences between distributions of descriptors attributed to identical numeric probabilities between scenarios, with more certain descriptors being used in scenario 2. **Conclusion:** Considerable inter-individual and inter-contextual variability exists in terms of how risk descriptors are applied to numeric probabilities for psychosis. For those providing counseling about risks for psychosis, in clinical practice, using risk descriptors only with caution may be appropriate.

EPL7.2 A population based study of the psychosocial impact of Klinefelter syndrome and attitudes towards diagnosis and screening

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Klinefelter Syndrome (KS) is a genetic condition (47XXY) affecting males, which results in a spectrum of clinical features including infertility, androgen deficiency, gynaecomastia, behavioural and learning difficulties. The prevalence has been estimated at 1:650, yet up to 70% remain undiagnosed. While the medical aspects have been explored, almost no evidence exists regarding the personal impact of KS. This study aims to determine (a) the psychosocial impact of KS (b) the influence on adult quality of life of age at diagnosis, testosterone treatments and other interventions, and (c) attitudes of men with KS towards optimal diagnosis age, acceptability of screening, and effectiveness of therapeutic interventions.

A population-based sample was recruited from a broad range of sources. 87 participants completed a written questionnaire. Of these, 79 provided a saliva sample for genetic analysis and 77 agreed to take part in an interview. The mean age was 43 years (range 19 to 76). Compared to population normative data, there was strong evidence ($p<0.001$) of the KS cohort having differences in: subjective well-being (PWI), body image (MBSRQ), self-esteem (RSE), and mental health (K-10). 72% of participants were diagnosed as adults, and 66% of these wished they had been diagnosed earlier. Two thirds of all participants supported population screening for KS.

For men with KS, there is a measurably negative psychosocial impact of having this condition. The possible benefits of achieving earlier diagnosis through population screening should be considered in relation to possible negative impacts, using available frameworks (Herlihy et al, 2010).

EPL7.3 The clinical utility of web-based familial risk information for diabetes prevention: do people adopt risk-reducing behaviours?

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Aim: To investigate how personalized prevention messages based on familial risk information using a web-based tool affect (determinants of) self-reported health behaviour (i.e. saturated fat intake and physical activity).

Methods: Overweight individuals with and without a family history of diabetes (aged 45 - 65 years) were randomized to receive web-based diabetes risk information based on: (1) general risk factors alone (control group, n=588), (2) general as well as familial risk factors (intervention group, n=586). Outcomes were assessed using questionnaires at baseline, post-test, and 3-months follow-up.

Results: There was no overall effect of familial risk information on health behaviour, except for low educated people a decrease in self-reported saturated fat intake was found ($p=0.05$). For people with a family history, there was no effect on the perception that heredity is an important cause of diabetes ($p=0.43$). In contrast, there was an increase of this perception for people without a family history ($p<0.01$). The intervention resulted in a lower increase in perceived susceptibility of diabetes for people with a family history ($p<0.05$). Whereas, no effect was found on perceived susceptibility for people without a family history.

Conclusions: People with a family history already seem to be aware of heredity as a cause of diabetes. Web-based familial risk information did not motivate risk-reducing behaviour, but might be effective for low educated people. Of note, the emphasis on familial risk information did not result in false reassurance for people without a family history, since there was no effect on perceived susceptibility.

EPL7.4 Parents', patients' and professionals' views on genetic counselling for cleft lip and palate

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Background: Individuals and parents of children with cleft lip and palate (CLP) may benefit from genetic counselling to discuss aetiology and recurrence risk. However, it is not clear what is the best way to

offer these services to achieve the balance between raising concerns amongst families at low risk, and providing risk information to those families who may benefit.

Aim: To explore the views of families affected by CLP, and of professionals involved in their care about how genetic counselling for CLP might best be provided.

Method: A qualitative approach was used, involving 3 focus groups (n=13), with healthcare professionals caring for families affected by CLP in Manchester (including speech therapists, surgeons and paediatricians) and 8 telephone interviews with patients and family members. Semi structured interview schedules were used that included questions about perceived advantages and disadvantages of attending a genetics appointment, preferences for receiving genetic information and understanding of roles within the CLP team. Transcripts were analysed using a modified grounded theory approach.

Results: Health professional participants had concerns about timing of referral, and uncertainties about how to approach the offer of genetic counselling. Patients wanted information from an 'expert' but felt the period immediately following the birth of an affected child was probably too soon. Patients and health professionals both wanted a clear plan for follow up, and access to information for specific groups e.g. teenagers.

Conclusion: Findings will be used to develop a model of service delivery for CLP families, for evaluation in a follow-up study.

EPL7.5 New European recommendations for genetic counselling - the views of clinical genetics professionals

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This study, carried out in collaboration with EuroGentest, sought to investigate practicing genetic professionals views of new ESHG en-

dorsed European recommendations for genetic counselling related to genetic testing (www.eurogentest.org). These guidelines outline pre and post test counselling for a range of situations including predictive, carrier and prenatal testing. Forty-six (response rate 35%) of invited genetic counselling professionals (25 medical geneticists, 25 genetic counsellors, and 4 psychologists) from 19 European countries participated, comprising a professionally and geographically diverse group of respondents all currently in clinical practice. A questionnaire including opportunities for free text responses was used to evaluate attitudes to the recommendations and relevance to their practice.

Participants were unanimously positive to having European guidelines. The most frequent reasons reported were: advantages to the professional of accessing collective expertise, promoting consistent quality of service provision, and providing a framework for developing local or national guidelines where this did not already exist. Ninety percent of participants reported that their current practice meets all or most of the recommendations, however half described areas for improvement, most commonly provision of written information to patients and adequate psychological support following test results. Lack of time and resources was the barrier to providing adequate provision in these areas and several respondents stated that genetic counselling had a low national priority leading to insufficient resources. Interestingly, very few professional or societal cultural barriers to implementing the guidelines were reported, although a few participants commented that recommendations could lead to a loss of professional flexibility and freedom.

Abstracts of EMPAG Concurrent Sessions

EC5.1 Genetic testing of newborns for type 1 diabetes susceptibility: A prospective cohort study on effects on maternal mental health

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Background

Genetic testing for susceptibility for type 1 diabetes was performed for a cohort of Norwegian newborns in the prospective study "Environmental Triggers of Type 1 Diabetes" (MIDIA). This study assesses whether mothers of the children that test positively suffer from poorer mental health and well-being after receiving genetic risk information about their children.

Methods

The study was based on data from the Norwegian Mother and Child Cohort Study (MoBa). Many of the mothers in the MoBa study took part in the MIDIA study where their newborn child was tested for genetic susceptibility for type 1 diabetes.

We used MoBa questionnaire data from the 30th week of pregnancy (baseline) and 6 months post partum (3 months post test). We measured maternal symptoms of anxiety and depression (SCL-8), maternal self-esteem (RSES) and satisfaction with life (SWLS). We compared questionnaire data from mothers who had received information that their newborn had high genetic risk for type 1 diabetes (N=166) with data from mothers who were informed that their baby did not have a high-risk genotype (N=7224). The association between risk information and maternal mental health measures was analysed using multiple linear regression analysis, controlling for baseline mental health scores.

Results

Information on genetic risk in their newborns showed no significant impact on maternal symptoms of anxiety and depression (p=0.9), self esteem (p=0.2) or satisfaction with life (p=0.2).

Conclusions

This study did not support that genetic risk information about their newborns has a negative impact on the mental health in Norwegian mothers.

EC5.2 Short-term psychological outcomes of ovarian cancer screening in women at high genetic risk

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Purpose: PsyFOCS is an ongoing multi-centre, prospective cohort study of the psychological effects of screening in women taking part in Phase 2 of the UK Familial Ovarian Cancer Screening Study. Phase 2 screening involves an annual scan of the ovaries and 4-monthly CA125 blood tests, with further tests prompted by receipt of either an intermediate blood result (rising CA125) or abnormal scan. These analyses examine the short-term outcomes for women who have received their first Phase 2 blood test result. **Method:** Women who received a normal result (n=799) and women who received an intermediate result (n=105) completed questionnaires at baseline and one week after receiving their results. The questionnaires included measures of cancer-related distress (Impact of Event Scale) and general anxiety and depression (Hospital Anxiety and Depression Scale). **Results:** There was a significant overall effect of time, with women reporting significantly greater levels of cancer-related distress one week after receiving a screening result compared to baseline (p<.001). However, women who received an intermediate result reported significantly greater increases in cancer-related distress compared to women who received a nor-

mal result (p<.001). No significant changes in general anxiety (p=.25) or depression (p=.64) were observed. **Conclusion:** These preliminary results suggest that participating in ovarian cancer screening leads to increased cancer-related distress in the short-term, but not increased general anxiety or depression. Future analyses will compare longer-term changes in distress and anxiety over time between women with normal results and women with intermediate results who are returned to routine screening following further tests.

EC5.3 Psychological distress in high-risk individuals undergoing pancreatic cancer screening

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Introduction In a research setting, individuals identified by clinical geneticists to be at high-risk for pancreatic cancer (PC) are offered PC screening (MRI and endoscopic ultrasound). However, it is yet unclear what the benefits, in terms of mortality are. This study investigates the psychological burden of, and experiences with, the PC surveillance program.

Methods Either mutation carriers of PC prone hereditary tumor syndromes (HTS; p16-Leiden, BRCA, p53, Peutz Jeghers Syndrome), or first degree relatives of patients with familial-PC (FPC) were invited to undergo PC screening. After screening, a questionnaire was sent assessing reasons for, and experiences with, screening, benefits and barriers of screening, concerns about cancer (Cancer Worry Scale), and anxiety and depression (Hospital Anxiety and Depression Scale). **Results** Forty-seven individuals (85%) completed the questionnaire (49% male, 26 HTS, 21 FPC). Most respondents underwent both EUS and MRI (94%). Most reported reason to undergo screening was: "Possible early detection of PC" (100%). Screening was reported as "very to extremely uncomfortable" by 13% for MRI, and 19% for EUS. Furthermore 32% worried most about the chance that relatives get cancer and 32% are "often" or "almost always" concerned about developing cancer themselves. Eight respondents (17%) have clinical levels of depression or anxiety. Almost all respondents (92%) reported that perceived advantages outweighed disadvantages of screening.

Conclusion Results indicate that one third of the respondents worry frequently about cancer, both for themselves and relatives. A minority seem to be in need of professional psychosocial care. However, the benefits of screening outweigh the barriers.

EC5.4 Applying measures of informed decision making to population carrier screening for fragile X syndrome

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Informed decision making (IDM) is important in order for individuals to make autonomous decisions without coercion or deception. Tools have been developed to measure IDM in prenatal Down syndrome screening, eg the multidimensional measure of informed choice (MMIC), plus a deliberation scale (MMIC+D). We are currently offering carrier screening for fragile X syndrome (FXS) to women from the general population and applying existing tools to examine IDM in this context. Women completed a questionnaire at the time of deciding about testing and provided a saliva sample (if tested). The questionnaire contained measures of FXS knowledge, attitudes and deliberation, as well as other questions derived from the Health Belief Model. The MMIC model was applied to these data, including that of test uptake. A subgroup of women were also interviewed. Responses in the structured interviews were compared with corresponding responses in the questionnaires to triangulate the findings. Interview responses were subjected to content analysis, coded by three independent researchers. Results are summarised in the table. 73% of women were classified as having made an 'informed choice' based on MMIC. Adding the deliberation scale resulted in 65% of women having made an 'informed deci-

sion'. While interview and questionnaire findings concurred, interviews highlighted factors other than poor knowledge, which contributed to the apparent lack of IDM. These factors are not captured by these measures, requiring different approaches.

| Questionnaire measures | N = 118 |
|---|---------|
| • Test uptake | 83% |
| • Good knowledge (K) | 81% |
| • Positive attitudes (A) | 87% |
| • Deliberated (D) * | 86% |
| MMIC - 'informed choice' (tested, good K, +ve A or not tested, good K, -ve A) | 73% |
| MMIC+D * - 'informed decision' (tested, good K, +ve A, D or not tested, good K, -ve A, D) | 65% |
| * N = 116 | |

EC5.5 Parental feelings towards the unsought identification of newborns as carrier of sickle cell anaemia through newborn screening

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In 2007, the neonatal screening programme in the Netherlands was expanded to include sickle cell anaemia. Besides identifying patients, this also leads to unsought identification of sickle cell carriers. Since the latter is not directly in the child's best interest, parents may 'opt-out' from receiving this information. The most important reason to disclose this information to the parents is to enable them to base their future reproductive choices on this. The aim of this study was to explore how parents perceive their child's carrier status being disclosed to them, and to see whether they take this information into account regarding their future reproductive choices.

Method

From March to September 2007, parents of newborns identified as sickle cell carriers, were invited to take part in a qualitative semi-structured face-to-face interview.

Results

48 parent-couples were invited, 19 accepted; eventually 13 interviews could be held. Parents were unaware of the possibility to 'opt-out' from receiving information about their child's carrier status. Initially, parents were shocked after receiving carrier information. Parents did not understand the proper meaning of carrier status. They had not understood that their child would not suffer from sickle cell anaemia. They

did not link their child's carrier status to their own future reproductive choices.

Conclusion

The way in which information on carrier status of sickle cell anaemia is being disclosed needs to be improved. The most important reason for disclosing this information - to enable the parents to make future reproductive choices - is not yet being achieved.

EC5.6 Knowledge and perceived risks in couples undergoing genetic testing after recurrent miscarriage or in men with poor semen quality.

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In reproductive medicine, couples with recurrent miscarriage (RM) and men with poor semen quality may undergo genetic testing as part of the diagnostic work-up. We explored the knowledge and perception of genetic testing in those couples, evaluated their psychological well-being and identified associated variables.

A prospective questionnaire study was conducted in 7 Clinical Genetics Centres and referring gynaecological departments in couples with RM or poor semen quality. Questionnaires were completed before disclosure of genetic test result. Main outcome measures were knowledge, perceived risk, anxiety and depression.

Almost 60% of participants (256 of 439) were not aware genetic testing was part of their diagnostic work-up. One third (36% RM, 33% poor semen quality) indicated they had not received information about the genetic test from their doctor. Perceived risk of receiving an abnormal genetic test result was higher than objective risk. Anxiety was highly correlated with perceived risk. Women with RM were more anxious than women in the poor semen quality group or men ($p<0.01$). There were no higher levels of depression.

Couples undergoing genetic testing after RM or with poor semen quality have a suboptimal understanding of the nature of testing, overestimate the risks of receiving an abnormal result, and some indicate high levels of anxiety. Improved knowledge of genetic testing in these couples is needed before a genetic test is performed. This could lead to more realistic expectations of patients about the consequences of the genetic test and to less anxiety before disclosure of the genetic test result.

Abstracts of EMPAG Workshops

EWS1.1 Teaching counselling skills for genetic counselling practice

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Teaching counselling skills for genetic practice is a core element of most genetic counselling programmes. In Europe there has recently been an increase in the number of Master level genetic counselling programmes with more due to start. The University of Manchester and University of Melbourne have been involved in teaching counselling skills to genetic counselling students since 1992 and 1996 respectively. A workshop is planned to discuss our combined experiences highlighting the need to balance theory with acquisition of skills, and to foster both relationship skills and reflective practice. This will be set against the competencies expected for professional certification. In addition we will demonstrate how new genetic counselling research evidence is integrated into the curriculum. Both programmes emphasize the importance of students having the opportunity to tape their own consultations/role plays, reflect on the process and receive feedback from their peer group and lecturer. The way role plays are set up for students and how feedback is provided will also be discussed. Excerpts from a prepared teaching videotape, using an actor as a simulated patient, will be shown. The second half of the workshop will include small group discussions with a facilitator to exchange views and ideas on methods of teaching counselling skills and theories considered relevant to genetic counselling practice.

EWS2.1 Embedding genetic counsellors into clinical genetics in Europe

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Across Europe there are wide variations in the roles and duties undertaken by genetic counsellors. In some countries, there are few genetic counsellors and no structure for involving them in clinical healthcare, while in other countries genetic counsellors have a formal registration system and are integral to the delivery of specialist genetic services. However, there is increasing evidence of the need for genetic counsellors to contribute to patient care in many contexts. For example, the guidelines on genetic testing developed by the EuroGentest project indicate that health professionals with specific training in genetic counselling should be involved in supporting patients in the pre- and post-test periods. In this workshop, we will explore the developing involvement of genetic counsellors in services across Europe. Perspectives from a range of cultural, national contexts and disciplinary contexts will be presented.

In the first session, patients who have experienced genetic services will contribute their perspectives on what the service user requires and expects from a genetic counsellor. The role of genetic counsellors in multi-disciplinary clinics and reference centres for rare diseases will be discussed by expert practitioners in the field of clinical genetics, while new and innovative approaches to embedding the work of genetic counsellors in other specialties will be presented by practitioners from Spain. Finally, the need to ensure patient safety through the use of counselling supervision for genetic counsellors will be explored. There will be opportunities for members of the audience to contribute their own opinions on the topic.

EWS3.1 Predictive testing for cardiogenetic conditions in children: practical considerations from a multidisciplinary team

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Background

Since 1996 we perform DNA-diagnostics for cardiogenetic conditions at our multidisciplinary outpatient clinic. In families with a pathogenic mutation predictive genetic testing is offered to relatives. Cardiac evaluations and/or treatment to prevent sudden death are advised to carriers. Familial sudden death often hurries relatives to get tested. Especially parents can feel powerless in their care and responsibil-

ity towards their children. Splitting up children and parents during the counselling may make children anxious. Counselling them together, however, gives little information on the psychosocial family background as parents may dread to speak openly. With this in mind we developed a counselling strategy with emphasis on support and protection of the children and their parents.

Methods and results

Our multidisciplinary team developed an informative booklet for parents with information on communication with their children on the genetic condition. Although this booklet helped parents to prepare their children, assessing the psychosocial family background remained difficult. Nowadays, parents receive the booklet and have a psychosocial intake by telephone with the psychosocial worker before the visit to our clinic. This provides us with enough information to tailor counselling to the needs and questions of the family.

Conclusion

By trial and error we developed a counselling strategy for families with children being tested for cardiogenetic conditions, which involves an informative booklet and telephonic consultation with a psychosocial worker before the counselling session. In a workshop we would like to discuss and present our strategy in more detail and exchange experiences to optimise counselling for these families.

EWS3.2 Cardiac chaos: A hypothetical about cardiac genetic services, issues and challenges.

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Follow the journey of the main character, Olivia, who, in this interactive hypothetical discovers that she has an inherited cardiovascular condition (ICC) in her family. Hear from an expert panel, how genetic counsellors, geneticists and cardiologists manage her problems and discover why researchers and ethicists have become involved. As genetic testing for ICCs such as long QT syndrome and hypertrophic cardiomyopathy becomes more common, genetics professionals need to be familiar not only with the medical and genetic aspects of these conditions, but also the unique challenges faced by families with ICCs. Sudden death, a small but real risk in these conditions, can occur at any time in individuals who are unaware of their risk status. Thus, the stakes are high for members of these families, which raises many dilemmas: are the usual strategies used by genetic professionals to facilitate communication about genetic risk sufficient in these cases? Could a more proactive approach be taken that honours patient confidentiality and respects individual autonomy? How should cardiac genetic services be delivered to meet these needs?

It is hoped that this hypothetical will stimulate discussion about different models of service delivery as well as the ethical issues and practical demands of ICCs on families and health practitioners. Audience participation will be encouraged to enable participants to compare and contrast approaches to the provision of cardiac genetic services around the world and reflect on counselling strategies that optimize outcomes.

Abstracts of EMPAG Posters

EP01.01 Genetic counselling for ambiguity: 46,XX/46,XY on chorionic villus sampling: what is the differential?

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A G3P2 couple was referred to the local maternity hospital for chorionic villus sampling (CVS) as a large nuchal fold was detected at 12 weeks gestation. A dating scan at 7 weeks gestation had confirmed a normal singleton pregnancy. Cultured CVS showed 46,XX[17]/47,XY[3], and FISH XX in 294 cells and XY in 86 cells in 3 separate culture flasks. Rapid aneuploidy screen on direct CVS showed no Y chromosome contribution. The couple was referred for genetic counselling to discuss the possible range of outcomes. Maternal cell contamination, vanishing twin, and chimerism were discussed with the couple, as well as the pros and cons of parental genotyping, fetal ultrasound sexing and amniocentesis to resolve uncertainty. The likelihood of maternal cell contamination was reduced by the finding of a female fetus on ultrasound. Amniocentesis showed 46,XX[10] and FISH XX on 500 cells, with female sex confirmed by molecular techniques. The couple was informed that an early vanishing twin was most likely responsible for the XX/XY CVS result despite the normal 7 week scan. These results have important implications about how we discuss accuracy of CVS, fetal sexing on maternal blood, and first trimester screening with our patients. This case illustrates the limitations of the technologies that we use and the challenges in explaining uncertain information to patients.

EP01.02 Counselling on reproductive options in Marfan Syndrome

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Marfan syndrome (MFS) is a progressive disorder with autosomal dominant inheritance and variable expression, that affects connective tissues. Cardinal manifestations involve the ocular, skeletal, and cardiovascular systems and strict criteria are used to establish the clinical diagnosis. MFS is caused by a defect in the fibrillin gene (*FBN1*) and mutations are found in \approx 80% of families with positive Ghent criteria. The search for these mutations is often driven by the reproductive risks and wishes of the affected individuals. This study was aimed at exploring the understanding of patients and their partners regarding reproductive risks and options, through questionnaires before and after counselling, in the setting of the Marfan clinic in Hospital Vall d'Hebron. A total of 36 individuals answered this survey and the major conclusions were: 1. Their understanding of the inheritance pattern, recurrence risks, medical risks of pregnancy and reproductive options were deficient even in those previously diagnosed, and improved markedly after counselling. 2. Those individuals who ignored their diagnoses or had not received appropriate counselling when they had their first offspring, showed anxiety and guilt. 3. Most individuals perceived the counselling as being useful and reducing their uncertainties and anxiety. 4. Every participant accepted prenatal and preimplantation (PGD) diagnoses as a method for avoiding having affected offspring. 5. Prenatal diagnosis with the option of termination of pregnancy was the first choice among most individuals, particularly in those looking for their first child. PGD was second. Globally, reproductive counselling appears to be essential in this group of patients.

EP01.03 PGD for cancer predisposition syndromes

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Introduction: Most cases of cancer are sporadic. Nonetheless, \sim 5-10% of the cases demonstrate an autosomal dominant inheritance pattern with variable penetrance. Prevention of unaffected offspring may be achieved by preimplantation genetic diagnosis (PGD). How-

ever, is PGD always justified?

Aim: To describe our experience in 41 PGD cycles for cancer predisposition syndromes that were approved by a Scoring System appraising PGD justification.

Methods: A semi-quantitative Scoring System was developed by evaluation of the disease characteristics (onset, severity, risk for offspring and penetrance) and patient variables (co-requirement for IVF, objection to TOP). PGD cycles were performed by blastomere biopsy of cleavage stage embryos or polar body biopsy, followed by single cell multiplex PCR that amplifies the cancer mutation and 3-5 flanking polymorphic markers.

Results: 17 couples applied for PGD for cancer predisposition. Fourteen couples were accepted according to our Scoring System and 3 couples were rejected due to under-threshold scoring values. Of the accepted couples, 9 were affected with cancer, 13 had at least one family member affected and 11 couples co-required IVF. In total, 6 pregnancies were achieved and 2 healthy children were born.

Conclusion: We envision that the continuous discovery of cancer predisposition mutations and identification of at-risk-families will result in an ever-increasing demand for prevention by PGD. We propose a Scoring System that takes into account the disease characteristics as well as the patients' clinical variables to determine which of these conditions and in which patients is PGD justified.

EP01.04 Choosing a hearing child: Attitudes towards the possibility of prenatal diagnosis for deafness in hearing parents of deaf children with cochlear implants

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Prenatal diagnosis (PND) of deafness in some families is now a theoretical possibility. At present, this is controversial and is neither routinely asked for nor routinely offered by genetics services. The majority of deaf children are born to parents with normal hearing, and it is these people whose decisions may be affected if PND for deafness were more widely available. This study investigates the attitudes of a subset of these parents whose deaf child has had a cochlear implant fitted. In opting for cochlear implantation, this group have actively chosen for their child to hear, so their views on PND are particularly pertinent.

These parents gave qualified support for PND for deafness, but were unanimous in their opposition to subsequent termination of pregnancy, the prevailing theme being that deafness was not serious enough to warrant such action. Their accounts demonstrate they are well informed and highly motivated to obtain the best possible treatment and support for their child, particularly with reference to participation in the hearing world. However, they describe their children as deaf, support their Deaf identities and do not perceive deafness as a serious disability. They have not 'chosen a hearing child', and are not interested in using genetic technology to ensure a hearing child in future pregnancies. Their point of view is closer to the cultural model than the medical model of deafness, which may be of some reassurance to those in the Deaf community who have viewed cochlear implantation as a threat to their future.

EP01.05 Psychological impact of preimplantation genetic diagnosis: A prospective Australian study.

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The psychological impact of preimplantation genetic diagnosis (PGD) was prospectively explored with fifty women from Sydney, Australia, using self-administered questionnaires. At pre-treatment (T1), anxiety and depression rates were similar to normal population data. State anxiety was associated with degree of financial worry and living in an inner metropolitan area. The women's perception of their chance of achieving a viable pregnancy were higher than the actual clinic rate and was positively associated with financial worry. The degree of financial worry may reflect the cost of PGD in Australia compounded by the

high cost of home ownership in Sydney, despite these women having on average high household incomes. The need for information about coping strategies if PGD was unsuccessful was identified. Unmet information needs were low but positively associated with women's education levels and information-seeking behaviours. Women completed follow-up questionnaires assessing anxiety and depression following embryo transfer (T2), pregnancy result notification (T3) and, for women who achieved a pregnancy, at 24 weeks of pregnancy (T4). Anxiety levels significantly increased following T2 and T3, though they returned to baseline levels at T4. Depression levels did not significantly fluctuate over time in this group. At T4, maternal-fetal attachment was assessed and found to be sound. A deeper understanding of these results was gained through additional in-depth interviews conducted with a subset of women (n=14). While describing PGD as stressful, women also reported PGD as empowering. Women reported previous reproductive trauma affected their PGD experience and their bonding with their fetus in early pregnancy.

EP02.01 Pregnant women's beliefs about prenatal testing for chromosome abnormalities.

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Current prenatal screening focuses on Down syndrome, whilst diagnostic testing gives information about all chromosomes. If noninvasive prenatal diagnosis for Down syndrome replaces current screening programmes, some non-Down syndrome chromosome abnormalities will no longer be detected. One way of minimizing reduced detection is inclusion of other chromosome abnormalities in noninvasive tests. Pregnant women's attitudes about appropriate inclusion may differ from that of experts and health professionals, as is seen with newborn screening programmes.²

The aims of our study are to determine pregnant women's opinions on which chromosome abnormalities should be included in prenatal testing and the beliefs underpinning those opinions. Using a health behaviour model based on the theories of Planned Behaviour and Reasoned Action, questionnaires were designed. A leaflet describing the outcomes of seven different chromosome abnormalities, including Down syndrome, was also provided.

The preliminary questionnaire elicits the beliefs from approximately 160 pregnant women. Using content analysis, the beliefs will be categorized into themes based on the main variables of the model; attitude toward the behaviour (positive or negative values towards the test), subjective norm (social pressure that may influence their decisions), and perceived behavioural control (what might get in the way of them actually having a test). The results will inform the main study questionnaire and ultimately assist in finding out what pregnant women want from prenatal testing for chromosome abnormalities.

References:

EP02.02 Acceptance of novel molecular techniques for prenatal screening of chromosomal alterations

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Pregnant women with an indication of prenatal invasive sampling due to risk for foetal anomalies, visited in Vall d'Hebron (Barcelona) and La Paz (Madrid) hospitals during 2009, were offered to take part in a study of the feasibility and cost-efficiency of novel methods for prenatal screening of chromosomal abnormalities (MLPA, aCGH), and a parallel study to identify factors influencing their acceptance of these screening tests. We provided pre-test genetic counselling to all women, including information on risks, expected detection rates and potential findings of unknown significance, as well as post-test genetic counselling when genomic alterations were detected. A short questionnaire about social and demographic characteristics and reasons to accept or refuse advanced prenatal testing was obtained.

The analysis of 450 surveys indicated that the great majority of women agreed to extend prenatal studies (94%). All women considered they had enough information, provided by pretest counselling, in order to

take decisions. The average anxiety level was in the intermediate-high range, mostly related to the indication for testing and previous history (previous offspring, assisted reproductive technologies, other...). The academic level of progenitors also influenced the reasons to participate and their understanding of the benefits and limitations of these techniques. Refusal of the study was related to anxiety, birth place and the indication for the invasive sampling.

In conclusion, our data show a high level of acceptance of new molecular techniques in prenatal setting and provide information about the most important aspects to discuss during genetic counselling concerning these novel molecular techniques.

EP02.03 Providing genetic risk information to parents of newborns with sickle cell trait: Role of the general practitioner in neonatal screening.

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In 2007 the newborn screening programme in the Netherlands included national screening of sickle cell disease (SCD). In addition, sickle cell trait (SCT) is identified and disclosed to parents of carriers. Since the introduction of this disclosure of SCT, general practitioners (GP's) are expected to play an important role in providing genetic risk information and refer at-risk couples appropriately. We aimed to investigate disease related knowledge, intention and motivational factors in order to evaluate this new role of GP's.

An assessment questionnaire was send in May 2009 to 285 GP's who received notice of a child with SCT in 2007 or 2008. The questionnaire contained questions on clinical experience and knowledge with SCD and SCT, communicating the result and potential barriers in the communication.

Response rate was 49%. Most GP's did not report barriers in counseling parents about SCT and providing risk information, even though the majority indicates they lack clinical experience with and knowledge about SCD and SCT. In our study only 23% of the identified couples at risk were tested for hemoglobinopathy, whereas 90% of GP's stated they informed parents. There was a great variation in knowledge of GP's in both the information they provided and the steps they took. This study shows that disclosure of SCT in the newborn screening at this moment only in a small percentage of cases leads to consecutive testing of parents for hemoglobinopathy. Much more effort is needed to provide better information to GP's and to help facilitate their work.

EP02.04 The changing landscape of genetic screening criteria

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In the past three decades in the Netherlands remarkable changes have occurred in population genetic screening. Governmental restraint in reproductive screening, based on an enduring emphasis on treatability as screening criterion, has given way to prenatal screening being available to all pregnant women, and neonatal screening has expanded dramatically, as in many countries.

Based on interviews, literature research, and results from a witness seminar, we will highlight three mechanisms that can put these changes into perspective.

1. Widening circles:

In the 1980s and 1990s the pros and cons of genetic prenatal testing on medical indication were known and weighed by a small circle of experts and health professionals. With the introduction of new techniques, making it possible to screen all pregnant women, wider circles (in society and Parliament) were confronted with ethical issues. Whenever circles widen ethical issues have to be assessed anew.

2. Differentiating between screening aims:

Where population screening is seen as a public health instrument (aiming for health gain by prevention or treatment through early detection) it is ill at ease with genetic screening providing reproductive options. Countries differ in their solutions to differentiate these domains.

3. Balancing protection and autonomy:

Traditional screening policy emphasized the protection of citizens against harmful or unsound screening, as is laid down in the Population Screening Act (1996). In the age of preventive genomics more tests and screening becomes available, and the demand for tests increases. These developments call for transparent alternatives to create a new balance between protection and autonomy.

EP02.05 POPULATION CARRIER SCREENING FOR FRAGILE X SYNDROME: CHALLENGES FOR GENETIC COUNSELLING

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Fragile X syndrome (FXS) is the leading cause of inherited intellectual disability. Carrier screening can provide individuals with information about their health and their risk of having a child with FXS. This study explored views about population carrier screening for FXS and here we discuss the relevance of these findings to genetic counselling. Interviews and focus groups were conducted with 141 participants: healthcare providers (81), relatives of individuals with FXS (29) and women from the general population who were offered carrier screening for FXS (31). Participants discussed issues relevant to genetic counselling including: challenges relating to decision-making; considerations about the most appropriate stage of life at which to offering carrier screening; the counselling needs of individuals who receive a grey zone, premutation, or full mutation result and perceptions of the role of genetic counselling in a population carrier screening program for FXS. The results indicate that there is support for preconception population-based FXS carrier screening, however, due to limited awareness of the condition, individuals from the general population will face unique challenges when making a decision about screening. Further, individuals should be supported to consider the best time in their lives to learn their carrier status. The most appropriate setting for offering screening was primary care, with genetic counsellors providing the service. These results will help inform future development of genetic counselling practice in this context and suggest that developing counselling protocols tailored to offering screening in the general population should be considered.

EP02.06 Haemoglobinopathy screening in the Netherlands: witnessing the past, lessons for the future.

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In The Netherlands there were no formal recommendations for haemoglobinopathy (HbP) screening until recently. In 2007 neonatal screening was expanded to include sickle cell disease. As a result carriers of this disease are also identified.

This study aimed to explore the decision-making process in the past: why had it been decided that HbP-screening was not 'opportune', and to investigate the role of ethnicity in the Dutch debate on haemoglobinopathy screening.

Methods: Literature search identifying key-figures involved in the decision-making process. They were telephone-interviewed, and invited to attend a so-called witness-seminar: i.e. elaborating on traditional sources of historical research, and adding new perspectives on past events. The transcript was content analyzed.

Results: During the 1980s and 1990s official screening policy was restrained regarding reproductive issues. Political parties expressed concerns about eugenics. However, locally screening practices did occur. In the nineties a research report requested by the government played a central role: Screening was considered not opportune due to low prevalence, and lack of knowledge amongst both professionals and high-risk groups. The heritage of WWII seemed to have influenced the decision-making process: registration of ethnicity surfaces as an important impeding factor.

Future issues for debate: Restrictive attitude to registration of ethnicity seems to have been relevant, but is changing as a result of increasing multi-ethnicity. The prevalence of haemoglobinopathies has increased. For future decision-making on haemoglobinopathy screening programmes, it is important to include representatives of high-risk groups in the discussion. These challenges will need to be addressed in policymaking.

EP03.01 Family construction of genetic meaning

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Testing minors to determine carrier status in X-linked disorders has generally been discouraged by professional organizations. One point of concern has been that of potentially harmful psychosocial impact upon the minor. Whether knowledge of carrier status will have a negative effect on minors, however, depends upon its meaning within the family system. Since testing is increasingly commercially available, health professionals now serve less often as gate-keepers, but can retain a valuable advisory role. Professionals who are asked to test the sister of an affected child can assess what implications a positive result might have within that particular family, being alert to the possibly great difference between parents' and daughter's perspective. Some parents may overestimate risk due to a cognitive error known as the availability heuristic (Tversky & Kahneman, 1973). This shortcut in reasoning makes unlikely events that have actually happened, and are therefore readily available to memory, seem more likely than objective estimates would indicate. Thus, parents' sense of the likelihood of grandchildren being born with a disorder that has affected a child of their own may be substantially elevated. Consequently, they may wish to protect their daughter, at all costs, from the ordeal they themselves have experienced, exerting undue pressure upon her to abandon hopes of child-bearing. Such parents who view the risk faced by carriers, or potential carriers, as overwhelming, need help in appreciating the dangers inherent in usurping control over future reproductive decisions that rightfully belong to their daughter.

EP03.02 Opening the psychological black box in genetic-counselling: The counselees' perception explains the psychological impact of DNA-testing, pathogenic-mutations the medical impact

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Background- It is often hypothesized that the Outcomes of DNA-testing (O) are better predicted and/or mediated by the counselees' Perception (P) than by the actually communicated genetic-Information (I). Method- Women tested for BRCA1/2 5 years before ($sd=2.0$), participated in a retrospective questionnaire study. Communicated Information was (I): cancer-risks and BRCA1/2-test result, i.e. unclassified-variant testresults ($n=76$), uninformative ($n=76$) or pathogenic-mutations ($n=51$). Four perception-variables were included (P): the counselees' recollections and interpretations of both the cancer-risks and the likelihood that the cancer in their family is heritable. The 25 outcome-variables (O) included life changes, medical decision-making, BRCA-self-concept, current psychological well-being and quality-of-life. Bootstrapping mediation analyses determined whether relationships were direct ($I \rightarrow O$ or $P \rightarrow O$) or indirect via the mediation of perception ($I \rightarrow P \rightarrow O$).

Results- Only the communication of pathogenic mutation or uninformative directly predicted medical-outcomes ($I \rightarrow O$), viz. performed and intended surgery. All other outcomes were predicted by the counselees' recollection and interpretation of their own cancer-risks and heredity-liability ($P \rightarrow O$), or this perception mediated all effects ($I \rightarrow P \rightarrow O$). However, this perception was significantly different from actually communicated cancer-risks ($I \rightarrow P$). The misperception of unclassified-variants predicted both psychological outcomes and radical medical decisions.

Discussion- Genetic-counsellors need to explicitly address the counselee's interpretations and intended medical decisions. In case of rigid misinterpretations additional psychological counseling might be offered. Communication of unclassified-variants needs close attention given the pitfall of ambiguity.

EP03.03 Overestimated lifetime breast cancer risk can be reduced by feedback on preexisting risk estimation.

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The majority of women with a family history of breast cancer estimate their lifetime risk (LTR) of breast cancer too high, even after genetic counseling. By discussing the counselee's estimation and emphasizing the actual risk during counseling, a more accurate LTR estimation is expected afterwards.

Counselees estimated their LTR in a pre- and post-counseling questionnaire. Their actual LTR was calculated using Claus tables and standardized breast cancer risk percentages. The intervention during counseling beholds a dialog on a counselee's LTR estimation and the actual LTR. This intervention was compared to standard counseling. Results confirm women in intervention and control group overestimate their LTR prior to counseling with an average overestimation of 43% and 42% respectively. Both groups showed a reduction of their overestimation after counseling. However the average overestimation of the actual LTR in the intervention group is significantly lower than the control group (11% to 31%, $p = 0.016$). In the control group 76% overestimate their LTR for at least 10% versus a significantly lower 36% doing so after intervention ($p = 0.034$). Conclusion: Integration of counselee's pre-existing risk estimations and feedback on actual LTR during counseling results in counselees estimating their breast cancer risk more accurately and therefore improved risk communication

EP04.01 Access to the Genetic Services in Cyprus

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¹Clinical Genetics Service, Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, ²Clinical Genetics Service, Makarios Medical Center, Nicosia, Cyprus. In the recent years the bicultural composition (Greek and Turkish Cypriots) of Cyprus has further diversified with the increase in "ethnically mixed" marriages (Cypriots with spouses mostly of Eastern-European and Asian ethnicity) and the increase in migration rates from both EU/non-EU countries. The Clinical Genetics Clinic (CGC), established in 1994, is the only reference center for clinical genetics and genetic counselling on the island. The CGC is therefore accessed by over 3500 patients and their families from all over Cyprus who are living with or are at risk of inheriting genetic conditions. The CGC offers diagnostic assessment, management/ treatment and genetic counselling in adult, prenatal, cancer and pediatric genetics for the whole spectrum of genetic conditions including chromosomal aberration syndromes, single gene disorders, mitochondrial diseases, multifactorial conditions and prenatal exposure syndromes. In accordance to changing population composition, the CGC-patient-population has shown a gradual increase in diversity. Patients/families may face challenges in access if i) language needs aren't met by the staff or a professional-translator ii) geographical location is unfavourable iii) religious/cultural beliefs (e.g emotional reactions/disbelief on genetic testing) are diverse iv) physicians knowledge/understanding of clinical genetics is insufficient v) low socioeconomic status vi) patient is from a 'paternalistic health care' culture, may view MD as the only expert over non-MD genetic counsellor. Such challenges are important to the members of the CGC who has to further enhance their cultural competence and adapt to the increasing diversity.

EP04.02 Dealing with Sudden Cardiac Death. A Comparison between Welsh (UK), Australian and North American Practice

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Genetic counselling for Inherited Cardiovascular Conditions (ICCs) is challenging to most Genetic Counsellors and Geneticists. Sudden unexplained/cardiac death (SUD/SCD) is a recognised (and often the first) presentation in Mendelian ICCs. The chapter 8 (Standard 5 for Wales) National Service Framework (NSF) in England for Coronary Heart Disease includes recommendations and sets out quality requirements, criteria and systems needed to provide diagnosis, treatment, information and support to families affected by SCD, in particular the sudden arrhythmic death syndrome (SADS). It states, 'When sudden death occurs, NHS services have systems in place to identify family

members at risk and provide personally tailored, sensitive and expert support, diagnosis, treatment, information and advice to close relatives'.

In Wales and parts of the UK there is a 'fragmented' approach to meeting the NSF quality requirements and recommendations due to (a) Inadequate funding and service infrastructure to meet the rapidly increasing referral numbers (b) New technologies are available enabling gene testing before patient pathways / protocols can be put in place (c) Multiple professionals being involved in SCD families, e.g. the investigation of the Coroners and Police may cause delay in referral of 'at risk' relatives for cardiac screening and clinical genetic assessment. A Florence Nightingale Travel Award to MN facilitated an observational study in Australia, Canada and Wales, to compare the delivery of clinical genetic services to family members affected by SCD/SADS.

EP04.03 Genetic counsellor: the Swiss experience.

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In Switzerland, genetic counselling is mainly provided by board certified clinical geneticists or oncologists. The medical genetic services of five university hospitals cover a population of 7.5 million inhabitants. A few private laboratories also provide genetic testing and counselling. Specific genetic counselling training is not available and genetic counsellors are not officially recognised by the Swiss Confederation. However, a step forward was made in 2008 when this profession was recognised in the Canton de Vaud, one of the 26 regional structures of the country.

We show the activity of three genetic counsellors working in the French speaking part of Switzerland and the evolution of their role. We also discuss the challenges and the aspects that have facilitated the development of this new profession.

Two genetic counsellors work in Geneva in cancer genetics. The third works in Lausanne in a general genetic counselling setting. We observe an evolution towards a diversification of the activity and a more autonomous role. The organisation of the clinical activity within the services, the lack of legal recognition and the lack of colleagues with the same background represent some of the challenges encountered. On the other hand, the establishment of a trust relationship with the colleagues and the hierarchy as well as the support from international networks have been helpful for the development of the role of genetic counsellor.

In the future, we will work towards a wider recognition of the profession in the country and the implementation of more genetic counsellor job positions.

EP04.04 Organisation and development of cancer genetics in Sweden.

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Purpose: The purpose of this study was to describe the organizational background and developments at cancer genetic centres in Sweden, as well as associated ethical issues.

Selection: Six cancer genetic centres were included in the study. In the initial phase the individuals in charge of their respective clinics were contacted and requested to select actors who participated in the development of the centres' operation.

Method: Qualitative content analysis was used to assess the material. The goal of content analysis is to provide knowledge and understanding of the phenomenon under study. Narrative content is analysed to discern themes and categories.

Conclusion: In summary, study findings showed that cancer genetics teams in Sweden need additional expertise. This line of work also requires constant ethical reflection to keep pace with technical and medical developments involving the human genome. As information related to these issues reaches the public through communication channels such as the mass media and scientific publications, expectations of health care resources are based on what published, and not what is technically possible and ethically justifiable. These considerations determine the planning and they shape the life choices made.

EP05.01 Declining genetic testing for hereditary breast cancer: psychological issues.

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Empirical studies show that only about 50 percent of women followed in high risk hereditary breast/ovarian cancer (HBOC) clinics opt to be tested.

The most common reasons for declining are concerns about the emotional impact, concerns about insurance issues, concerns about the absence of a concrete benefit on surveillance, worries about the limitations of screening and concerns about test accuracy.

To our knowledge, all studies so far which have investigated the emotional functioning and motivations of test decliners included only individuals enrolled in HBOC clinics and thus were focused on a relatively selected group of persons who all received genetic counseling or took part in a surveillance program. People who are anxious or unfavorable about testing are not likely to participate in this type of research.

In the presented study we specifically aim at interviewing people who are unknown to the genetic center. Recruitment of individuals is done through a tested family member who is willing to participate. We use semi-structured interviews and qualitative data analysis (IPA).

The study is ongoing and so far, recruitment has proven to be difficult. Preliminary results indicate deeply rooted fatalistic beliefs and doubts about the idea of manageability.

Investigating these psychological issues can also be important in the light of a more general debate about the role of genetic services in disclosing genetic information within families.

EP05.02 Debating genetics with young people: the GAMY (Genetics and Merthyr Youth) Project

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Purpose: Young people's opinions about genetics are rarely heard, although they are most likely to be affected by advances in genetic knowledge. The GAMY (Genetics and Merthyr Youth) Project worked with teenagers from South Wales over a two year period to explore how confident they felt about discussing genetics and investigate their attitudes.

Method: Participants (aged 16 to 19 years old) engaged in four group days involving learning and discussion about genetics topics including inheritance, risk, family history, genetic testing and ethical issues. They completed genetics tasks, expressing their ideas through creative media such as digital photography and storytelling. A mixed methods approach was used to collect a variety of data from all aspects of the project.

Results: 21 young people were recruited, though participation declined over time. They had learned about genetics at school, but did not understand how to relate this to their own lives. Through involvement with the project, most became more confident to discuss genetic issues and their understanding increased, although they retained some misconceptions and their opinions can be resistant to change. In general, they were interested in genetic advances, though found it difficult to appreciate the potential psychosocial impact.

Conclusions: With appropriate support and information, young people become more genetically literate, and acquire the skills and confidence to discuss genetics issues. Further research is needed to explore how genetic literacy can be promoted more broadly within this age group to enable them to be involved in debates about genetic developments.

EP05.03 Perceptions of Genetic Counseling from Adults with Bipolar Disorder

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Bipolar disorder is a mood disorder that affects about 1% of the population. Twin studies provide evidence that genetics plays a role in the etiology of bipolar disorder with heritability estimates as high as 93% in some studies. Molecular genetic studies were initially promising, but no genes with large effect sizes have been discovered. There is also evidence that suggests a multifactorial inheritance pattern. Genetic testing is not yet available. However, as advances are made in the understanding of genetics and psychiatric disorders, it is expected that

the demand for genetic counseling for bipolar disorder will increase. Genetic counselors are well-equipped to educate patients and family members about the condition, to discuss their concerns about the risk of bipolar disorder, and to offer genetic testing should it become available. For this qualitative study, interviews were conducted to explore the opinions and perceptions of individuals with bipolar disorder and/or their siblings. The open-ended questions were designed to elicit the thoughts and attitudes about bipolar disorder and genetic counseling. Thematic analysis was performed on the transcripts from 12 interviews. Data analysis is ongoing, but the preliminary analysis reveals some common themes. These include: 1.) a perceived need/interest for a diagnostic test, 2.) excessive disease burden on patients and family members, 3.) individuals sometimes use their family history to make reproductive planning decisions, 4.) the causal attributions for bipolar disorder vary among individuals, and 5.) an interest in genetic counseling to learn more about bipolar disorder.

EP06.01 Psychological follow-up after disclosure of cancer genetic test results in BRCA 1/2 mutated families

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Objective: To describe the psychosocial support of BRCA1/2 carriers/non carriers by two years after test result delivery.

Methods: French national cohort of unaffected carriers/non carriers (N=622) within BRCA1/2 families. Questionnaires including medical, psychosocial (IES, CES-D, BIS, social support...) and socio-demographic characteristics were completed before and 15 days, 12 months and 24 months after test result disclosure.

Results: Answer rate (N=533; 86%, 40 years old on average; SD=12). Psychological referral has been offered to 72% and 32% of carriers (n=232) and non carriers (n=301) respectively (p<0.001). After multi-variate adjustment, carriers were more frequently offered referral when they were younger, when a psychologist attended at the result consultation, and when they had a higher psychological distress. Among the non carriers, the level of distress was the only factor correlated to psychological referral. By one and 2 years, 20% of the carriers attended at a psychological consultation, compared to 8% of the non-carriers (p<0.05). Related factors will be detailed.

EP06.02 Predictive genetic testing in adolescents for late-onset conditions: First-hand accounts from minors

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Due to advances in genetic technology, asymptomatic individuals at-risk of genetic conditions can undergo predictive testing (PT) to find out if they will develop the condition later in life. PT is routinely offered to adults at-risk of late-onset conditions (eg: Huntington's disease, BRCA, HNPCC). More controversial is the application of PT in minors for late-onset conditions where no effective treatment or prevention is available. The ethical discourse concerning PT in minors is lively, yet it remains one of speculation, with a relative scarcity of empirical evidence. While sporadic reports of PT in minors and the rise of the 'mature-minor' doctrine have led to slightly greater flexibility in recommendations worldwide, most maintain a general position against PT in minors for conditions without effective medical intervention.

This study employed a qualitative methodology to explore young people's experience of being tested for an adult-onset genetic condition (including a range of cancers and neuorogenetic disorders). It entailed semi-structured interviews with adolescents and their families. Ten interviews were conducted. Interviews were transcribed and thematically analysed to reveal a range of harms and benefits associated with participants' PT experience, many of which were unrelated to the actual test result.

Through the presentation of detailed case-studies, this paper will describe a range of harms and benefits associated with PT in minors. For the first time this study gives a voice to the adolescents who are the centre of this debate, provoking consideration of current guidelines and questioning whether we have reached the correct balance in existing recommendations.

EP06.03 Quality assessment of genetic counselling process in predictive testing for late-onset disorders: What we know?

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Little is known about the impact of the amount and quality of genetic counselling on the psychosocial sequelae of genetic testing for late-onset disorders. Although some guidelines have been written regarding number of sessions, involvement of multidisciplinary teams and topics for pre-test discussions, more understanding about what helps consultands prepare effectively for testing is needed.

We conducted a systematic review of papers reporting reviews on the quality of genetic counselling aiming at: 1) exploring the current evidence available; 2) identifying quality assessment indicators for the genetic counselling process in late-onset disorders; and 3) proposing some recommendations for genetic services to improve the quality of health care. After quality appraisal of the papers thus identified, four were included.

Our preliminary findings revealed that research on counselling in the context of genetic testing for cancer tends to be related to outcomes and process indicators for quality assessment. Research concerning testing for other late-onset diseases is mainly focused on the psychological impact of the results, but much is insufficiently articulated regarding quality and content of the whole process. Number of sessions, time spent, consultation environment, follow-up, multidisciplinarity, among others are identified as measures for quality assessment.

However, despite the fact that presymptomatic testing for disorders such as Huntington disease and other degenerative conditions has been offered for more than 20 years, good methodological approaches to assess the quality of genetic counselling for predictive remain elusive. This restricts improvement of the protocols for genetic services and in general health care for the at-risk population.

EP07.01 Tracking coping responses over time with The Genetic Risk Assessment Coping Evaluation (GRACE).

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Objective: To develop and evaluate the effectiveness of a cancer-ge- netic specific measure of coping (The Genetic Risk Assessment Coping Evaluation (GRACE).

Method: GRACE measures the degree of stress associated with 11 stressors for individuals undergoing cancer genetic risk assessment, and the use of up to eight coping strategies they may elicit.

Results: 194 patients completed GRACE upon referral to the All-Wales Medical Genetics Service and one month later whilst awaiting genetic risk information. The most highly endorsed source of stress at both time points related to the implications of risk for family members, endorsed by 72% of patients at Time1 and 57% at Time 2. Patients made use of multiple coping strategies across different sources of stress. There was a significant decrease over time across all stressors in information-seeking (23.9% to 14.7%) and emotional expression (13.2% to 8%). Positive appraisal remained one of the most commonly used coping strategies across all stressors ranging from 24%-45% at Time 1 and 16%-47% at Time 2.

Conclusions: Responses suggested that patients were using appropriate coping strategies for the type of stressor faced. The completion rates for the matrix and specificity of responses provided suggests the GRACE may be an acceptable and useful measurement tool and may also help genetic counsellors identify individuals in need to extra support.

EP07.02 Development and testing of a screening questionnaire for psychosocial problems in genetic counseling and testing for cancer.

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Introduction: Approximately 20% of all individuals undergoing genetic counseling and testing for cancer experience significant psychosocial problems during or after the counseling process. However, in approximately 30%, of these cases, such problems remain undetected. The aim of this study is to develop and validate a psychosocial screening questionnaire for use in genetic counseling for cancer.

Methods: EORTC Quality of Life Group guidelines for questionnaire development are being used to develop the questionnaire. This involves four phases: 1) generation of relevant issues, 2) operationalization of these issues into a set of items, 3) pre-testing, and 4) larger scale field testing.

Results: To identify relevant problems, we carried out a literature search and interviewed 26 experts and 5 former counselees. Twenty-six issues were identified and operationalized into items covering the following six problem-areas: family, children, genetics, cancer, practical issues, and emotions. The screening questionnaire has been transferred to a web-based platform with touchscreen computer facilities in the clinic. In total, 120 counselees will be invited to complete the screening questionnaire, along with the Hospital Anxiety and Depression Scale and the Distress Thermometer. The questionnaire data will be compared with data obtained from a semi-structured interview conducted by an experienced psychosocial worker ("gold standard"). Conclusion: This project will yield a screening questionnaire that can aid in systematically identifying counselees with specific psychosocial problems in cancer genetics. Subsequent to validation, a trial will be carried out to investigate the effectiveness of the screening questionnaire in facilitating communication and triggering appropriate counseling and referrals.

EP07.03 "I have always believed I was at high risk..." The role of expectation in emotional responses to the receipt of a cancer genetic risk assessment result: A thematic analysis of free-text questionnaire comments.

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Background: The receipt of cancer genetic risk information can evoke a mix of both positive and negative emotional responses. Objective risk itself is not necessarily predictive of emotional response to receipt of risk information and the Cue Adaptive Reasoning Account (CARA; Renner, 2004) suggests that that the degree to which level of risk is consistent with expectations may influence emotional responses.

Method: Free-text questionnaire data was analysed from 123 women and 15 men, after receiving their cancer genetic risk assessment result. A thematic analysis was structured around spontaneous responses to the three risk labels: average, moderate or high.

Results: Reactions to risk information appear to be dependent upon participants' pre-conceived expectations about their level of cancer risk, often due to family or personal history of cancer. Many average risk respondents questioned the accuracy of their result, whereas high risk information was often expected.

Discussion: The current findings provide support for the CARA model, as receiving a high risk result may have been consistent with expectations and therefore did not evoke an extreme emotional response in many respondents. The CARA model provides a theoretical explanation for the negative reaction from respondents at average risk, as unexpected risk information may require high cognitive effort and a tendency to downplay the accuracy of the information.

EP07.04 An online screening decision aid for men with a family history of prostate cancer: development and pilot-testing

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Background: Men with a family history of prostate cancer have unmet information needs relating to how best to manage their cancer risk. This study aimed to develop and pilot test an online decision aid (DA) tailored to men with a family history of prostate cancer to assist them to make an informed choice about screening.

Methods: Men aged 40–79 years with a family history of prostate cancer were invited to participate in the study by a letter conveyed to them by an affected male relative who attended a urology outpatient clinic. The DA was evaluated by eligible men in two stages. First, men evaluated the acceptability of a paper-based version of the DA in a questionnaire (N = 22). Second, the same men were asked to appraise the functionality of an online version of the DA and to provide their feedback in a telephone interview (N = 20).

Results: Ninety-one percent of participants reported that they found the paper-based DA useful, and that it contained enough information to make a decision about screening (73%). All men reported that the online DA was easy to use, with the majority reporting that they would recommend the website to other men. Most men also reported that they would prefer to receive information about screening via a website rather than in a booklet (78%).

Conclusions: This study provides a valuable insight into the optimal design and distribution of information about prostate cancer screening for men who are at increased risk of this disease.

EP07.05 Women's decisions to withdraw from a familial ovarian cancer screening programme

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Background: A prospective psychological evaluation study (PsyFOCS) is underway in partnership with a familial ovarian cancer screening study (UK FOCSS, Phase 2). The screening study aims to detect ovarian cancer at an early stage when treatment is most effective, using 4-monthly CA125 blood tests and annual scans. Cross-sectional data are presented describing the factors associated with women's decisions to withdraw from screening.

Methods: 1,390 out of 2,395 women completed a baseline questionnaire. Measures included cancer-related distress (Impact of Event Scale), anxiety and depression (Hospital Anxiety and Depression Scale), and illness representations (adapted Illness Perceptions Questionnaire-Revised; IPQ-R), along with a free text section. 77 women withdrew from screening before their first Phase 2 blood test.

Findings: The main reason women withdrew from screening was to have their ovaries removed to reduce their risk of developing ovarian cancer. Logistic regression indicated that previous experience of requiring repeat tests for non-normal screening results was associated with withdrawal from screening ($p<0.01$), as was testing positive for a gene mutation known to increase the risk of ovarian cancer ($p<0.05$) and greater cancer-related distress ($p\leq0.05$). Marginal associations were found for greater IPQ-R illness coherence ($p=0.08$) and marital status ($p=0.09$). Thematic analysis of free text data highlighted factors such as the practicalities and emotional impact of screening, and strat-

egies for coping with risk.

Discussion: Withdrawal from familial ovarian cancer screening may be influenced by both clinical and psychological factors. This may reflect women's clearer understanding of ovarian cancer risk and the pros and cons of screening.

EP07.06 Genetic counseling and hereditary cancer. A prospective study with emphasis on psychosocial aspects

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Background: During the last few decades there has been an increased demand for genetic counseling (GC). This trend is expected to continue. Thus, we need more knowledge of patients undergoing GC and tools that can identify those in need of extra attention from the counselor, and those who will manage with an ordinary counseling session.

Aims: The overall aims of the present study were to describe and study the course and outcome of GC for hereditary cancer and to identify characteristics of vulnerable subjects, in order to tailor and increase the efficiency of the GC process.

Methods: A prospective multicenter study was undertaken among subjects undergoing GC for hereditary breast- ovarian cancer and hereditary nonpolyposis colorectal cancer.

Results: On the average, the participants reported high levels of social support, GC-specific self-efficacy, general self-efficacy and physical functioning. The average levels of anxiety, depression was low and cancer-related intrusion and avoidance was moderate. Both anxiety and depression declined over time and both more social support and GC-specific self-efficacy predicted lower anxiety and depression after GC. Subjects reporting lower level of GC-specific self-efficacy and high level of worry were vulnerable to both intrusion and avoidance.

Conclusion: The majority of the subjects undergoing GC for hereditary cancer were coping well. Still, we identified a subgroup that seems to be more vulnerable during follow-up. They were characterized by less social support, lower GC-specific self-efficacy, higher level of worry, and by young age. In the years to come a tailored, individualized counseling approach may be necessary.

EP08.01 The views of health professionals regarding the management and care of families affected by sudden arrhythmic death syndrome

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Purpose: Intense media and public pressure led to the inclusion of sudden death related to a genetic cause in the UK National Service Framework for heart diseases. However, there is no agreed process for providing adequate support to families affected by sudden arrhythmic death syndrome (SADS). **Method:** Focus groups were carried out with health professionals and patient representatives in Wales to explore views on the current management of families affected by SADS and how services could be improved. **Results:** Focus group participants described the often ad hoc nature of current service provision, the difficulties in informing families about the possibility of inherited risk in a sensitive and timely manner, and lack of awareness of SADS in people potentially working with these families. Family members were described as having different information needs at different stages in their dual process of grieving and adapting to genetic risk. Possible solutions proposed by focus group participants centred on the need for an adequately resourced, uniform approach combining cardiology and genetics. Key components of this approach included having a dedicated coordinator who could work proactively across professional boundaries, raise awareness of SADS, and take an individualised approach with families; recognition of the therapeutic nature of encounters with families; and access to specialist bereavement counselling where appropriate. **Conclusions:** As cardiac genetic knowledge increases, families affected by SADS are increasingly likely to benefit from referral to joint cardiology and clinical genetic services. A coordinated, sustainable approach would meet the complex, evolving needs of families affected by SADS.

EP08.02 The psychosocial impact of a complex genetic results in a family with Hypertrophic Cardiomyopathy (HCM).

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Screening for mutations in 4 of the 8 genes associated with HCM is available clinically and identifies mutations in 70-80% of cases. The use of genetic testing in HCM families has helped to clarify individuals' risks and to guide clinical management including cardiac screening. There is increasing recognition of the importance of digenic inheritance in inherited cardiac conditions, however, when an additional unclassified variant is identified this can increase uncertainty for the family. We present a case where a variant of uncertain significance, in addition to a known pathogenic mutation, was identified which highlights the difficulty in communicating complex results to the family and the psychological impact. A 45 year old man with HCM was tested. His results showed a known pathogenic mutation in the MYH7 gene and an unclassified variant in the MBPC3 gene. The variant had not previously been reported, but is predicted to result in an amino acid substitution leading to an exchange of an uncharged polar amino acid with a non-polar amino acid, at a position conserved across some species, suggesting the potential for pathogenicity. With these provisos, cascade screening in the next generation was initiated. The results raised several issues for the family: i) the change in dynamics within the family with the carriers being more protected, ii) anxiety caused by cardiac screening for the asymptomatic children and iii) uncertainty due to the variability in phenotype in the context of two variants.

EP09.01 Attitudes toward hypothetical genetic testing, satisfaction with clinical care, and information needs among high risk melanoma survivors: A mixed methods study

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Background: A previous diagnosis of melanoma plus multiple dysplastic naevi, or multiple primary melanomas constitute a 10 to 20-fold risk of developing further melanomas; a risk comparable to that of individuals with a strong family history of the disease. Currently, genetic testing for melanoma risk is not available. The High Risk Clinic (HRC) at the Sydney Melanoma Diagnostic Centre provides a specialist service dedicated to the treatment and follow-up of high-risk melanoma survivors. The aim of this mixed methods study was to explore high-risk melanoma survivors' attitudes toward hypothetical genetic testing, satisfaction with clinical care at the HRC, and their support and information needs.

Methods: Twenty patients at increased risk of developing further melanomas, who had attended the HRC, were invited to take part in a semi-structured telephone interview. Information preferences were assessed with structured questions at the end of each interview.

Results: Participants appraised their experience of clinical care positively with most reporting that they felt well informed and supported. More information regarding the role of genetics and moles in the development of melanoma was identified as an unmet need. Despite limited knowledge of genetic risk assessment for melanoma, 70% of participants endorsed hypothetical genetic testing. A consultation with an expert or a pamphlet was the preferred source of information about melanoma.

Conclusions: High-risk melanoma survivors expressed satisfaction with the clinical care received at the HRC and more than half endorsed hypothetical genetic testing. The development of tailored written information in addition to expert clinical consultation is warranted.

EP09.02 Finding contentment after prophylactic interventions in women with BRCA1 or BRCA2 mutations.

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Purpose: To describe the decision-making process for women who are BRCA1 or BRCA2 mutation carriers and choose to undergo a prophylactic operation.

Method: A qualitative cross-section analysis in which eleven women are interviewed who have undergone genetic screening for cancer at Sahlgrenska University Hospital in Gothenburg, Sweden. The material has been subjected to qualitative content analysis.

Results: The decision-making process is part of a time flow in which various components stand out as important conditions that enable decisions to be made about prophylactic operations:

Threat against life, which comprises both a subjective threat in the form of the woman's familial history and an objective threat in the form of the results of the genetic testing.

Time - a space for manageability, a meaningful time during which both internal and external resources provide support for the woman in her process.

The experience and insight that the woman is at a crossroads where she has an opportunity to make a choice, which means that she is given *the opportunity to choose life*. Many times there will be a specific event that has a very strong effect on the woman and facilitates the decision to have a prophylactic operation.

Finding contentment is the theme that deals with where the woman finds herself today, and gives expression to what has come of the process.

EP10.01 Communication of genetic information by other health professionals: the role of the genetic counsellor in specialist clinics.

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Many children with chronic genetic diseases are followed by specialty clinics who address the family's genetic issues as part of the child's care. Health services restrictions can make accessibility for an appointment with a genetic counsellor difficult. We examined whether genetic information was being adequately understood when presented by medical, but non-genetics staff to long term patients, using our National metabolic service as an example. Little research has been carried looking at communication of genetic information with metabolic conditions. This study aimed to inform health professionals about the need or role of a genetic counsellor in specialist settings. We investigated knowledge about recessive conditions and the reproductive impact of the information in patients with Galactosemia and Maple Syrup Urine Disease (MSUD) and their parents. Adult patients and parents of affected children were interviewed in person using a questionnaire to focus on disease and genetic knowledge of these conditions. Parents showed a good level of knowledge but there were misunderstandings about the risk or implications of carrier status. Adult patients with Galactosemia had more misunderstandings in relation to inheritance, recurrence risks and carrier status than their parents. We found that there was a statistically significant difference between adult patients and their parents for Galactosemia and between Irish and Irish Traveller parents. The knowledge difference between parents and adults may reflect a reluctance to transmit genetic information within families. Results suggest that patients with inherited conditions under long-term care of other health conditions could still benefit from input from a genetic counsellor.

EP10.02 Impact of genetic testing in couples with recurrent miscarriage or with poor semen quality: anxiety and distress 3 and 12 months after disclosure.

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Most research on the consequences of genetic testing is performed in persons with an increased risk for a specific disease (pre-symptomatic

subjects) but not in patients already affected by disease. In reproductive medicine, couples with recurrent miscarriage (RM) and men with poor semen quality may undergo genetic testing as part of the diagnostic work-up. It is unknown if psycho-social outcomes are influenced when receiving an abnormal genetic testresult in these couples. We conducted a prospective index-control questionnaire study in 7 Academic Medical Centres at the departments of Clinical Genetics and their referring gynaecological departments. Questionnaires were sent to both partners of couples with RM or poor semen quality (between 2006-2009). Questionnaires were completed before disclosure, and 3 and 12 months after disclosure of the genetic testresult. Main outcomes were anxiety and depression. Anxiety was measured with the State Trait Anxiety Inventory and depression with the Beck's Depression Inventory. Participants in whom a genetic abnormality was found were compared to participants in whom no genetic abnormality was found. Questionnaires were returned by 439 participants (222 men and 217 women), 172 participants with RM (39%) and 267 participants with poor semen quality (61%). A genetic abnormality was found in 36 couples with RM and in 48 couples with poor semen quality. At the time of submission of this abstract, the database is being finalized, with closure scheduled on April 1st 2010. Definite data on the 3 and 12 month follow-up will be presented at the EMPAG meeting in June 2010.

EP10.03 The family secret in Cyprus. Health professionals' accounts of family communication for Huntington's disease.

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Facilitating risk communication among HD families is challenging for health professionals in Cyprus. Given the availability of options such as PND and PGD, it is crucial that families at risk are informed by the proband. This paper presents an illustrative case of how cultural and institutional practices in Cyprus have shaped current patterns of risk communication.

This paper investigates factors that influence family communication in Cyprus and how health professionals experience such factors in clinic. Health professionals working at the Cyprus Institute of Neurology and Genetics were interviewed in 2009. Semi-structured interviews (n = 5) were conducted in Greek. Data was translated, transcribed and analysed using thematic analysis and grounded theory.

Cultural factors such as discrimination, over-protectiveness, family duty and the value of "philotimo" greatly influence communication and decision making in Cypriot families. Professionals perceive their responsibilities and roles differently according to their background and training. For instance, neurologists tend to focus on medical options and hold a public health perspective, while professionals with psychosocial training were more client-centred, less directive and less judgemental. Elements of medical paternalism were identified and linked with health professional-client relationship. Existing structural and organizational problems increase the burden on health professionals and cause mistrust towards the health system.

Cultural awareness and understanding of client experience within health care settings might help professionals who work with HD families. Psychosocial counselling and unconditional positive regard are important when dealing with clients who have been exposed to discrimination and paternalism.

EP10.04 Familial Hypercholesterolaemia: the challenge of implementing the NICE guidelines.

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Clinical guidance from the National Institute of Clinical Excellence (NICE) have recently made recommendations regarding the identification and management of Familial Hypercholesterolaemia. The key priorities for implementation include DNA cascading testing if the family mutation is known.

Familial Hypercholesterolaemia (FH) is an autosomal dominant disorder with a prevalence of 1 in 500, approximately 110,000 people are thought to be affected in the UK, but unfortunately the majority remain undiagnosed. Effective preventative treatment is available, if untreated it leads to premature Coronary Heart Disease (CHD) and death. Cas-

cade testing using genotyping has recently become available in South Wales on a research basis.

The main aims of this study were to explore how patients and their family members receive, make sense of and transmit genetic information and the impact this dynamic process has on perceptions of risk. Face to face semi structured interviews were conducted with seven patients in whom a clinical diagnosis of FH had been made and who had recently undergone genetic testing and received a mutation positive result. A further seven interviews were conducted with members of their families who had also undergone genetic testing to follow the flow of this genetic risk information. The interview transcripts were thematically analysed to gain insight into their experiences.

The findings suggest that this genetic information help the patients to make sense of their condition. Family members reported an open style of communication although this process and emotional responses to genetic risk information were complex.

EP10.05 Tactile Diagrams to improve communication with patients with severe visual impairment in the genetic counselling clinic.

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Genetic counselling for inherited blindness presents communication challenges. Traditionally, genetic services use diagrams to explain inheritance patterns and recent evidence also suggests that the "personalised diagram" helps families put the scientific information into the context of their family experience (Gale T et al, 2010). Patients with a visual impairment (VI) are not able to access traditional drawings or diagrams and are therefore disadvantaged in the genetic counselling clinic. Within the UK, it is our legal obligation to provide equal access to services for patients with disability (UK Disability Act, 1995) and therefore we are undertaking a project to evaluate communication tools within genetic ophthalmic service including summary letters and leaflets in audio files and email and computer technology for enlarging diagrams in clinics.

Patients with a severe visual impairment require tactile diagrams to explore pictures of inheritance. However, we do not know the acceptability to the patient, the skills needed by the clinician or which diagrams may be most useful. Tactile diagrams for inheritance patterns were developed using focus groups composed of visually impaired patients and representatives from specialist organisations for people with visual problems including Action for Blind People and the Royal National Institute for the Blind (RNIB). We will present outcomes from the focus groups and the use of tactile diagrams in the genetic clinic which reveal how these might be successfully introduced in clinical practice. We hope to show how these communication aids improve communication with VI patients in the genetic clinic with minimal cost or effort.

EP10.06 Knowledge about hereditary nonpolyposis colorectal cancer: mutation carriers and physicians at equal levels

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Background: Identification and adequate management of individuals at risk for hereditary nonpolyposis colorectal cancer (HNPCC) is crucial since surveillance programmes reduce morbidity and mortality. We investigated knowledge about key features of HNPCC in at risk individuals and physicians in surgery, gynecology and oncology.

Methods: Data were collected using a questionnaire which was answered by 67 mutation carriers and 102 physicians from the southern Swedish health care region. The statements were related to colorectal cancer, heredity and surveillance and the physicians were also asked questions about cancer risks and surveillance strategies.

Results: Both groups answered questions on colorectal cancer risk, surveillance and genetic testing well, whereas answers about inheritance and risks for HNPCC associated cancer were less accurate. Only half of the family members and one third of the physicians correctly estimated the risk to inherit an HNPCC predisposing mutation. Among family members, young age (<57 years), female sex and recent genetic counseling significantly correlated with better results. Physicians generally underestimated the risk of HNPCC associated cancers and three out of four suggested a

later starting age for surveillance than recommended.

Conclusion: The finding of similar levels of knowledge about key features of HNPCC in at risk

individuals and physicians reflect the challenge physicians face in keeping up to date on hereditary cancer and may have implications for the clinical management and professional relations with HNPCC family members.

EP10.07 Factors influencing the uptake of genetic counselling in female relatives of people with haemophilia.

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Since 1988, about 200 people with haemophilia (PWH) and their families have been seen by genetic counsellors at the Haemophilia Care Centre at Charlotte Maxeke Johannesburg Academic Hospital. However, very few of their at-risk female relatives have attended genetic counselling to discuss their reproductive risks and options or their potential bleeding risks. Minimal research has been conducted internationally or in South Africa around the factors influencing uptake of genetic counselling and testing amongst female relatives of PWH. This prospective qualitative study aims to: (a) determine what factors influence obligate and potential carriers' uptake of genetic counselling and testing, (b) determine what would assist subjects in managing their carrier status, and (c) assess what would help these women to obtain genetic counselling and testing.

An open-ended semi-structured interview schedule was developed to incorporate potential predictors of genetic counselling and testing uptake as well as recommendations for improved carrier care and genetic service. Subjects include female relatives of PWH who at least have a family member who has been counselled. Content analysis will be used to identify themes in the 20 subjects interviewed.

Preliminary findings indicate that some of the deterrents to uptake of genetic counselling and testing include: misunderstanding its purpose, fear of knowing carrier status, lack of knowledge about reproductive risks and potential bleeding symptoms.

The information gained from the research will be used to provide a better service to female relatives of PWH; with a goal being to set up a dedicated clinic for these women.

EP10.08 Factors influencing uptake of cascade carrier testing by adult relatives after a diagnosis of cystic fibrosis through newborn screening

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In Victoria, Australia, carrier testing for cystic fibrosis (CF) is performed by a state-wide laboratory and counselling service. CF carrier testing (cascade testing) is offered free to relatives of babies diagnosed with CF through newborn screening. Although cascade testing is known to detect carriers for CF, its effectiveness has been questioned because most babies with CF are born to couples who do not have a family history. Uptake of testing was audited by examining pedigrees of newborns diagnosed with CF, and performing data linkage to the laboratory database records. Uptake of carrier testing amongst adult relatives was adjusted for clustering within families; three relatives per family, other than the parents, have had carrier testing. The majority of relatives have not had cascade carrier testing despite being at high risk. We identified factors influencing uptake of CF carrier testing by administering a questionnaire to a sample of relatives, and interviewing parents of children with CF. The response rate was 79% (n=225). The results of logistic regression analysis are summarised in the table. Qualitative data from the questionnaire and interviews indicate the main barrier to testing was lack of awareness that cascade testing was available through the clinical service. Findings provide insight into the characteristics of relatives who are tested and those who are not, and inform clinical service delivery around offering carrier testing to family members.

| Variable | Proportion tested | Unadjusted analysis OR (95% CI) | Adjusted analysis OR (95% CI) |
|-------------------------------------|-------------------|------------------------------------|----------------------------------|
| Gender of relative | | | |
| <i>Female</i> | 42% | 1.8 (1.1 to 3.1) | 2.0 (0.9 to 4.3) |
| <i>Male</i> | 29% | reference group | reference group |
| | | p=0.02 | p=0.07 |
| Category of relationship | | | |
| <i>Parent</i> | 53% | reference group | reference group |
| <i>Aunt/uncle</i> | 53% | 0.9 (0.4 to 2.3) | 1.4 (0.5 to 4.2) |
| <i>Grandparent</i> | 35% | 0.4 (0.2 to 1.1) | 0.6 (0.2 to 1.5) |
| <i>Other</i> | 16% | 0.1 (0.0 to 0.3) | 0.2 (0.1 to 0.7) |
| | | p<0.001 | p=0.01 |
| Have children already | | | |
| <i>Yes</i> | 40% | 2.6 (1.5 to 4.5) | 2.9 (0.9 to 8.7) |
| <i>No</i> | 21% | reference group | reference group |
| | | p<0.001 | p=0.06 |
| Plan to have (more) children | | | |
| <i>Yes</i> | 30% | reference group | reference group |
| <i>No</i> | 38% | 1.3 (0.5 to 3.2) | 0.4 (0.1 to 1.7) |
| <i>Currently pregnant</i> | 75% | 5.7 (1.0 to 33.0) | 2.5 (0.0 to 123.6) |
| <i>Don't know</i> | 43% | 1.6 (0.5 to 5.4) | 0.9 (0.1 to 7.1) |
| | | p=0.1 | p=0.4 |
| Knowledge score | | 2.7 (1.8 to 4.2) | 2.0 (1.1 to 3.8) |
| | | p<0.001 | p=0.03 |
| Attitude score | | 1.5 (1.2 to 2.0) | 1.3 (0.9 to 1.8) |
| | | p=0.003 | p=0.1 |

EP10.09 Quality assessment of genetic counseling with focus on prenatal diagnoses -Bilateral survey of counseling staff and clients-

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Objective: Tokai University Hospital is a core regional medical facility with 800 beds offering advanced, and emergency medical care. Genetic counseling was started in 2007, and geneticists, nurses, and clinical psychologists provide counseling to approximately 200 clients/year (2009), regarding prenatal diagnoses (90%), congenital abnormalities, and genetic disorders. Counseling quality was surveyed in the largest body of clients seeking prenatal diagnoses and medical staff, in the interest of improving service quality.

Method: Clients and two attending staff (physician and nurse) were asked to complete an 8-item 5-point Likert scale—the Genetic Counseling Satisfaction Scale (adapted from K.P. Tercyak, 2001) directly after counseling. Client-version questionnaires carried additional items addressing age, objective/reason for seeking counseling, accompanying persons, extent of prior knowledge, waiting/consulting time, decision-making, and budgetary concerns. The study was conducted from March 2009 through January 2010. Responses were obtained from 64 clients and 128 staff members.

Results: Most clients were in their 30s, and 60% of the consultations were age related. Following counseling, 33% elected to undergo amniocentesis, 8.6% against, with the remainder postponing decisions pending further consultation. Mean client Satisfaction Scale scores for items ranged between 4.3-4.7, higher than staff scores by 0.5-1.7 points. Lowest evaluation by clients (4.3 points) was for „Readily understandable explanations,“ and among staff, „Explanation of basic knowledge“ (2.8 points). Client comments requesting information on fees, test schedules, and need of genealogical data when making the initial appointment indicated their wish to approach the consultation well prepared.

EP10.10 Adaptation of a cancer communication skills training model for professional development in genetic counseling: description and evaluation

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Ongoing professional development for genetic counselors is critical in maintaining best practice in knowledge and skills. Communication skills training (CST) workshops for doctors and nurses in oncology to provide counseling skills, utilizing trained actors in role plays with facilitator and group feedback, have been implemented for many years in Australia. This model has been adapted in the provision of continuing professional development in counseling skills for practicing genetic counselors in Australia since 2002, providing a unique opportunity for reflection and focus on practice rather than teaching new skills. Pre-workshop, actors worked with a director to develop their characters and explore responses in scenarios designed to illustrate issues derived from reported challenges in genetic counseling practice. Evaluation of participants' experience and impact included surveys following immediately post the one day workshops (2002, 2004, 2005 and 2008 (x2); n= 88/100) and 2-5 years later, (2007; n=21/38) and a focus group with previous participants (2007; n=7). All rated the workshop as effective methodology for professional development. Aspects highly valued included facilitator feedback; actors rather than role-playing with peers and so being able to stop and try things differently; group feedback; addressing new challenges; reassurance about their own practice and "material to work on in the workplace". Perceived outcomes on practice included the opportunity to reflect, bring focus to communication, increase motivation and confidence. The high level of satisfaction is a strong endorsement for ongoing communication skills training in this format as part of professional development for genetic counselors.

EP11.01 Facilitating decision making and providing support in the face of uncertainty

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Many families pursue genetic counselling in the hope of obtaining a greater level of certainty around the condition in the family, the risks to them and their children, and information about possible options to manage these risks. Genetic testing aims to help provide answers to some of these questions. However, in some cases testing fails to provide a confirmatory diagnosis. Genetic counselling in these circumstances can be challenging. Individuals may find it more difficult to make decisions in the face of uncertainty. This can leave both patients and professionals feeling powerless.

Here we present 2 cases illustrating the difficulties inherent in facilitating decision making in the absence of a confirmed genetic diagnosis. 1) A couple presenting at 9 weeks of pregnancy whose previous daughter died aged 3 months from Severe Combined Immunodeficiency (SCID) where genetic testing has not identified a causative mutation. 2) Two sisters in their early twenties concerned about a strong family history of young onset breast cancer where no mutations in BRCA1, BRCA2 or p53 were found.

We describe the counselling techniques and models utilised in these cases to assist the decision making process and consider the impact of these scenarios on the families.

EP11.02 Cross-cultural values of 'informed choice' in antenatal genetic screening

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Policies for UK antenatal genetic screening programmes state that their primary aim is to facilitate 'informed choices'. These policies are largely guided by Western ethical principles of autonomy but implemented within a multi-ethnic population. Building on the evidence for cultural differences with respect to patient autonomy, this is the first study to explore values of informed choice within a multiethnic population within the context of antenatal genetic screening. The study used Q methodology to characterise understandings of informed choice in a multiethnic sample of postnatal women. Ninety-eight participants of African, British white, Caribbean, Chinese and Pakistani origin completed a 41-statement Q-sort, which was also translated into French,

Mandarin and Urdu. Statements reflected different values of informed choice in antenatal screening in terms of understandings of the test; the role of health professionals in decision-making; social influences; autonomy; structural factors; values of diagnostic testing, termination of pregnancy and religion. Q-Factor analysis produced statistically independent viewpoints of the value of informed choice. These were interpreted using comments participants made in post-Q-sorting interviews. The findings show that women hold a variety of views of the nature of 'informed choice', and that, contradictory to policies of non-directiveness many women seek and value the 'advice' of health professionals. The findings have implications for the role of health professionals in facilitating informed choice, quality of care and equity of access.

EP12.01 Case study: Herlitz junctional epidermolysis bullosa. Genetic counselling challenges in a highly consanguineous new migrant family.

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This case study involves a large Iraqi family with multiple consanguineous loops with a devastating family history of Herlitz junctional epidermolysis bullosa (H-JEB). H-JEB is a rare autosomal recessive skin disorder, usually fatal in infancy. It is characterized by skin fragility and blistering, nail inflammation; granulated wounds particularly on the face and a hoarse cry due to laryngeal involvement. The defective protein is laminin 332, a key component of the junction of the epidermis and dermis. Mutations in four genes have been described in JEB with the LAMB3 mutation accounting for 70% of all cases, including this family.

The family believes at least 30 babies have died with this condition in the past 100 years and until 2008 the diagnosis was not known. The psychosocial issues in counseling this large extended family were complex and challenging. All communication was with an Arabic interpreter. Interviews with the women in this family revealed they: feared for every pregnancy, grieved for previous losses, experienced guilt from the perception of passing on faulty genes, had their religious beliefs challenged, and struggled to make reproductive choices.

A Swedish branch of the family was the first to have mutational analysis and following this Dr Jonasson and associates in Sweden provided mutation testing on the Australian branches of the family. The mutation was found in the LAMB3 gene in exon6 (c.430C>T) introducing a stop codon p.R144X. Four branches of the family in Australia have now had mutational analysis for carrier diagnosis and reproductive options.

EP12.02 How often is disclosure of genetic information important in genetic counselling?

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Section 95AA of the Australian Privacy Act addresses the potential need for health professionals to disclose genetic information to relatives, even without consent, when there is a "...serious threat to life, health or safety of their genetic relatives...". These guidelines (October 2009) provide examples of how this might eventuate.

We are undertaking a RCT of a genetic counselling intervention to facilitate communication of genetic information within families, expecting that disclosure issues would be prevalent amongst the clientele in our population.

Four participating clinics were asked to audit their clients for approximately 3 months to determine the number eligible for recruitment i.e. there was a need for disclosure of genetic information within the family. If they were ineligible, the genetic counsellors were asked to provide a reason.

There were 513 clients attending the clinics and 27 (5%) clients who were eligible for recruitment in the audit period. Seven exclusion categories emerged:

| Exclusion category | Not index case | Prenatal testing | No family implications* | Review | Results pending | No genetic diagnosis | Other# |
|--|----------------|------------------|-------------------------|----------|-----------------|----------------------|---------|
| Number of clients (% of total clients attending clinics) | 91 (18%) | 106 (21%) | 82 (16%) | 72 (14%) | 33 (6%) | 86 (17%) | 19 (4%) |

* e.g. sporadic chromosome mutation, teratogen, de novo mutation, condition may not be genetic
e.g. interpreter required, cancer patients, genetic counsellor not present, hereditary haemochromatosis, client already recruited, parent with an intellectual disability.

In only 5% of genetic counselling sessions was disclosure of genetic information to relatives found to be applicable. As previous studies have indicated that instances of non-disclosure within families are rare, in the professional experience of a genetic counsellor the magnitude of the need for disclosure without consent is going to be extremely small; although where they arise the Section 95AA guidelines can now be used.

EP12.03

Communication of risk in families living with Huntington's disease: a web survey to explore what they think, what they do.

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Huntington's disease (HD) is a neurodegenerative, autosomal dominant, late-onset disease neither curable nor preventable.

We explored parental practices of informing children of risk for HD and the differences, if any, in truth disclosure from a generation to the other. An anonymous Internet survey was proposed to visitors from HD families on the lay Association website www.aichroma.com.

The survey explored the following issues:

- The way they, as children, had been informed of risk for HD
- The best age to receive the information and the ideal provider
- Whether being informed is useful or not
- The way they, as parents, had informed (or planned to inform) their children

Eighty-five individuals responded: 80 were HD families members and 5 partners.

Preliminary data show that parents have difficulty communicating children about disease and risk. The majority of respondents had children in condition of risk: about 80% of children had a parent who was carrier or symptomatic or at risk. Among respondents, those who did not have children stated they would inform them in case they had while the majority of those who actually had children did not provide any information nor showed any clear intention of doing it in the future.

Telling children the truth about HD and risk raises ambivalence. The opportunity to access good sources of counseling and support seems relevant in order to enable families to manage the emotional distress of giving such information.

EP12.04 Ethical aspects of genetics: testing asymptomatic minors: our experience

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When a genetic disorder is diagnosed other family members, including minors, can also be at risk for the disorder. For many medical issues parents give informed consent for their children. In genetic testing of children there are some special points of interest. It has to be considered what the advantages are of testing during childhood. An important factor is the possible onset of the disorder in childhood and the opportunity to decrease the risk of health problems with a specific periodic control scheme. The choice of the moment of testing depends on several factors. If there is an adult onset of the disorder there are less advantages of testing in childhood. A comparable issue is test-

ing of carriership of a balanced chromosomal translocation in childhood. Then the preference is to give the children their own choice to know their genetic status in adulthood. As long as they are minors counsellors have to consider that the child is not (fully) aware of all the consequences of the test-outcomes. Of course there are exceptions, there are some reasons to test during childhood on a disorder with adult onset. Preferably this decision is made by parents and the child together. Psychosocial support during and after the test period is important. We give some examples of testing of (adult-onset) genetic disorders in childhood.

EP12.05 Experiences in men belonging to a family where women have a risk to fall ill with hereditary breast and ovarian cancer.

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Introduction: At present, focus is mainly on the women in families undergoing screening for familial breast cancer and/or ovarian cancer. This is only to be expected, since this involves the risk of developing cancer diseases primarily in the female organs. The family being screened have often experienced the devastation of the family when female relatives have died, which is perceived as an imminent threat. The men in the family are often contacted after confirmation of a mutation in the family. The men may thus be carriers of the mutation and pass it on to any children.

Purpose: The purpose of the literature review was to gain deeper understanding of how men think and feel when they may be carriers of a known mutation, aimed at expanding the communicative arsenal for genetic counsellors / nurses in their encounters with these men.

Method: The material included in the literature review was analysed using content analysis and the findings presented in two themes: decision-making and communication.

Findings: The findings are reported in sub-themes, which clarified how the family influences men in their decisions prior to carrier testing. These are related to worries about their own health as well as thoughts about whether their carrier status implies a responsibility to refrain from reproduction. Men also have personal experience of cancer through their mothers, sisters and daughters. The study findings reveal the key role of the genetic counsellor / nurse in the human encounter and communication with men in the healthcare setting.

EP12.06 The baby who was not born after a prenatal diagnosis: the un-thinkable hole in the family's net.

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In this presentation I hope to show how prenatal diagnosis (PND) can be revolutionary for the couple and family dynamics. Since 2001, I have accompanied in the Genetics Department more than 200 couples who went through termination of pregnancy (TOP) for medical reasons. Drawing from these clinical cases, we will see how the experience of TOP is a *de-meshing* attack on the couple's mesh, and how a psychotherapeutic intervention assists in the family's *re-meshing* (Benghozi, 2009, 2005).

The techniques of PND take place in the most dynamic phase of the baby's representation. Although the baby is still unborn, at this stage future parents are organising a new *intersubjective link* (Benghozi, 2009, 2005) with the baby, as are other family members. In a word, broadening of the familiar net is already taking place. Could the experience of TOP tear the mesh that unites the couple and family?

The baby who was not born becomes a hole in the family's net, a "ghost" with an *un-thinkable*, *un-nameable* and *un-confessable*¹ representation. This may explain why women often go alone to the therapeutic sessions. This "ghost" bears also the power to haunt the family's capacity to dream of the next baby, the one not yet conceived but who already carries the risk of *inheriting a family's psychic material which could not be elaborated by the previous generations* (Benghozi, 2005).

Psychotherapeutic intervention should help the couple make this hole nameable and go through the work of re-meshing the intersubjective links of the family's net.

EP12.07 Familial breast cancer: Incongruence between the understanding of an inconclusive test result and informing relatives at risk.

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Objective: In many families with a strong history of breast cancer no mutation in the BRCA1/2 genes is identified. In these families, relatives may nevertheless need to be informed about their increased risk and screening advices. Little is known about this group and the aim of this study was to explore index patients' perspective on whether relatives at risk were informed, to what extent relatives were informed and which difficulties they experienced when transmitting risk information.

Methods: Semi-structured interviews were performed by telephone with eighteen index patients who had attended genetic counselling. Transcribed interviews were analyzed qualitatively.

Results: Nearly all first degree relatives at risk were informed about the test result and their screening advice, second and third degree relatives were not. Most participants interpreted the test result as 'truly negative'. In some cases this misunderstanding led to non-disclosure because according to the index patient there was no or little risk. However, in others cases it facilitated disclosure because no mutation being found was seen as a reassuring message. Several other factors influencing the process of disclosure emerged, including the degree of contact with relatives, the sense of responsibility to inform and the attitude of relatives towards genetic testing.

Conclusions: Informing relatives about their increased risk for developing breast cancer is influenced by many factors. It appeared that implications of inclusive test results are complex and difficult to understand. Despite this, nearly all first degree relatives were informed. Second and third degree relatives, however, were not.

EP13.01 Development of a new measure of adaptation to living with a genetic condition or at risk.

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We propose a new scale for measuring adaptation to a genetic condition or risk. Living with a genetic condition or risk often leads to stress that patients learn to cope with. Adaptation refers to the process of coming to terms with the health threat and the observable outcomes of that process. Definitions of adaptation and synonyms like "adjustment" and "acceptance" differ in the literature. A variety of scales have been used to measure these related concepts. There is need for an adaptation scale based upon stress and coping models that captures both intrapersonal and interpersonal outcomes of the process. Based on the theoretical and empirical evidence, we identified four primary domains of adaptation: coping responses to the condition, self-esteem, social relationships, and the search for spiritual or existential meaning. Using items available in the PROMIS "positive outcomes of illness" item bank, we constructed a scale with five items for each of the four domains. The scale has been used in six studies of adaptation to: Huntington disease risk (n=191), a child with pervasive developmental disorder (n=324), neurofibromatosis (n=482), a child with Down syndrome (n=546), XXY syndrome (n=249), and a child with Rhett syndrome (n=400). The Cronbach alpha measures of reliability exceed 0.8 in each study and confirmatory factor analysis suggests that each set of five items converge on a single domain. Use of the scale is intended to lead to comparisons across different populations of individuals living with a genetic condition or at risk.

EP13.02 Epidermolysis Bullosa - exploring the experiences of affected individuals and families of living with an inherited skin condition

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Epidermolysis Bullosa (EB) is a rare inherited skin disease, affecting at least 5000 people in the UK. It is a genetically and clinically heterogeneous disorder, which encompasses a broad spectrum of symptoms of varying clinical severity. As found in many other skin diseases it may

also have significant associated psychosocial issues, which add to the already challenging role faced by those affected by the condition and health professionals. A review of recent literature revealed limited published qualitative work on the psychosocial needs and experiences of individuals and families affected by EB, particularly those affected by the more common but generally less severe form of the disease, EB Simplex. This study involved semi-structured interviews with 7 people with a personal or family history of EB who were members of DebRA. The verbatim transcripts were thematically analysed to identify important and common issues. The issues raised were varied, which was to be expected, given the complex and multi-faceted nature of the condition. These were divided into two main themes of 'awareness' and 'burden of illness', which included both physical and psychological challenges, such as pain, appearance, visibility and quality of life issues caused by the constant need to plan ahead and adapt. The theme of awareness covered issues such as education, knowledge amongst health professionals and other people's awareness of EB. The experiences and attitudes of those affected by EB are vital to broadening our understanding of this complex condition and improving treatment and care of families.

EP13.03 EXTERNAL VALIDATION OF THE HEREDITARY NONPOLYPOSIS COLORECTAL CANCER SELF-CONCEPT SCALE IN THREE COUNTRIES

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Purpose Hereditary nonpolyposis colorectal cancer (HNPCC) renders high risks of colorectal and gynecological cancer and implies life-long surveillance. Knowledge of an increased risk of cancer offers possibilities for prevention, but may also influence self-perception and increase psychological distress. A 20-item self-concept scale has recently been developed with the aim to capture HNPCC-specific psychosocial difficulties. We assessed the external validity of this instrument, using data from three HNPCC populations in Denmark, Canada, and Sweden. Methods Self-concept scores were available from 591 individuals with HNPCC-predisposing germline mutations. The subgroups included 415 Danes, 108 Canadians, and 68 Swedes without differences in sex and age between the populations and with 43-63% being affected with cancer. ANOVA analyses were used to test for differences in the subgroups and principal component analysis was used to explain variance.

Results Overall, the HNPCC self-concept scale provided similar results in the three countries, through differences were recognized for individual questions. Danes expressed lower degrees of guilt, but more pronounced worries for cancer, whereas Canadians more often expressed feelings of isolation and loss of privacy. PCA analysis demonstrated that all 20 items contribute to variability with questions related to gastrointestinal anxiety being major determinants.

Conclusion The HNPCC self-concept scale was found to be valid and reliable. Subtle differences in feelings of guilt, worries for cancer, isolation, and loss of privacy were identified between the countries.

All items contributed to the variation in scores with items linked to gastrointestinal anxiety central.

EP13.04 A review of the literature comparing the burden of disability and social factors on living with Charcot-Marie-Tooth or Myotonic Dystrophy

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The burden of living with a progressive neuromuscular condition has received very little attention in the literature. A total of 15 papers were identified as satisfying the inclusion criteria that examined the burden of living with Charcot-Marie-Tooth (CMT) or Myotonic Dystrophy (DM). The majority used quantitative research methods to measure quality of life (QoL), while one paper used qualitative methods. CMT is classed as a relatively mild condition with variable but often late onset. DM also has variable onset but is associated with increased cognitive and physical impairment which becomes worse in successive generations. In the papers examined, higher levels of disability were found to correlate with decreased QoL. In these progressively deteriorating conditions it is often assumed that as the individual gets older, and the disease progresses, that QoL would deteriorate. This is not always the

case. A "response shift" (Bostrom and Ahlstrom, 2004) is thought to occur between expectations and reality, making disability easier to cope with over time, despite the progression of the condition. Interestingly in DM, social factors were found to have a greater impact on QoL than level of disability. Lower levels of education and employment were observed in DM compared to CMT. Lack of education or employment was found to negatively influence QoL. The study identifies areas of concern for patients that have relevance in clinical practice in Genetic Medicine, as well as possibilities for future research in this subject.

EP13.05 Huntington Disease - A practical CD-guide

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Background: Huntington disease is rare and knowledge about the disease varies among professionals. A person affected by the condition who needs healthcare often meets professionals who lack adequate knowledge and may have to inform the professionals about Huntington disease. Within affected families many questions arise, especially among children at risk of inheriting the disease.

Aim: The purpose was to create and disseminate easily accessible, detailed resources about Huntington disease, directed to professionals, the affected, and to families.

Method: We made a detailed description of the progressive illness in five phases, connecting the different needs in each phase to special training, healthcare and social services. This was sent to all Huntington disease teams in Sweden for comments, which were incorporated into the resource. Researchers and persons with connections to Huntington disease were interviewed and the recorded interviews were included in the final resource, a CD-Rom.

Result: The resource was presented at the national Huntington disease meeting in January 2010 but was already well known by the specialist teams through their participation in creating the CD. We are spreading information about the CD through patient organizations and media. The anticipated outcome is that this resource should fill the knowledge gap for professionals and affected families.

Future plans

- Write a guide for professionals who work with affected patients in daily care.
- Create an e-learning program from the resource material for professionals.
- Develop information for children in different ages.
- Translate the CD to English through the Euro-HD-network.

EP14.01 Development and evaluation of a telephone counselling strategy to enhance family communication about genetics

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Individuals who receive a diagnosis of a genetic condition are generally advised to inform at-risk family members of this information. However, many family members appear to remain uninformed about their risk status. The responsibilities of health professionals towards these family members and ethical approaches have been debated but with little resolution.

Instead, we focus on the affected individual and present a client-centred genetic counselling strategy to facilitate family communication of genetic information. This can be delivered by telephone and complements face to face consultations. Development of the strategy was iterative and informed by empirical research about family communication. Counselling and communication theories were drawn on in order to optimise clinical feasibility and maintain congruence with the tenets of genetic counselling.

The process of evaluation of the strategy and the training protocol for genetic counsellors will be described. We will reflect upon the ethical challenges presented and the ways in which these were resolved.

The efficacy of the intervention is currently being tested by means of a randomised controlled trial funded by the Australian National Health and Medical Research Council. (NHMRC).

EP15.01 Statutory Regulation for Genetic Counsellors in the UK.

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Professional registration for UK genetic counsellors began in 2001 and over half of the UK genetic counsellors now have a voluntary certificate of registration. Regulation of health care professionals has been prominent on the UK government agenda over the last few years and the resulting White Paper, "Trust, Assurance and Safety: The regulation of health professionals" (2007) highlighted the need for public protection and proportionate regulation for new and emerging health professions.

Within clinical genetics, doctors and clinical scientists are registered with statutory regulation bodies such as the General Medical Council (GMC), genetic counsellors are not statutory regulated with growing numbers of non-regulated MSc trained genetic counsellors and nurse trained genetic counsellors now working within a new scope of practice. The Association of Genetic Nurses and Counsellors formed a steering group in 2006 to prepare an application for statutory regulation to the Health Professions Council (HPC). In order to join the HPC, professions must have evidence that they are an autonomous, stand-alone profession with evidenced based practice, a code of conduct, defined entry level criteria and demonstrate a registration process with fitness to practice and commitment to continuing professional development. We will present the case for statutory regulation of genetic counselling, the importance of public protection, and the professional achievements in the UK, which lead to the approval of genetic counsellors by the HPC in December 2009. The profession is now awaiting the government's consideration, including public consultation, for the necessary changes in law for statutory regulation in the UK.

EP15.02 Genetic counsellors' perceptions of the blurred boundaries between clinical research and clinical care

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As genetics embeds itself in mainstream medicine, the degree of specialisation of genetic counsellors is growing and we are finding ourselves with increasing opportunities to be involved in research as part of our everyday clinical care.

We recognise that research improves our clients' lives, and that our involvement in research helps to develop genetic counselling as an independent profession.

Research and clinical care are theoretically distinct due to their differing motivations, aims, and outcomes. However in practice, these activities can become blurred in the eyes of both the clients and the genetic counsellors, creating a sense of unease for both groups for differing reasons.

This study uses an online survey to investigate practising genetic counsellors' experiences in introducing clients into, or actively recruiting them for, clinical research. It aims to uncover some of our apprehensions about this process in the hope that our awareness of the issues can help us distinguish these two parts of our roles, enhancing the overall care for the client in an ethical and responsible way.

Some of the concerns include the appropriateness of timing of recruitment (soon after or during clinical care sessions), the feelings of coercion associated with development of client rapport, the ethical dilemmas associated with having incentives to recruit clients to projects, and being unable to ensure our clients are aware of the distinction between the clinical and research activities.

EP15.03 A pilot study of music therapy in the treatment of children with developmental delay

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Music therapy is known as an anxiolytic and musical play promotes non-verbal interaction and dialogue, specifically enhancing the prosodic elements of communication.

Goal: To assess what changes occur in developmentally delayed children when treated with active music therapy.

Methods: A crossover study of music therapy in twelve developmentally delayed patients were assessed, selected and randomly allocated into two groups. Each child received individual music therapy.

This formed a treatment group and an initial non-treatment group to serve as a waiting-list control. The non-treatment group received music therapy after waiting for three months, while the previously treated children had a break from therapy. The main assessment was developmental according to psychological and functional criteria (the Griffiths' test), and musical.

Results: The children in the initial treatment group change more than the children on the waiting list. Such changes can be demonstrated at a level of clinical significance ($p < 0.05$, Cohen's $d = 1.7796$).

When the waiting list group are treated and then tested, the newly treated children catch up in their development. There is a continuing improvement in hearing and speech, hand-eye co-ordination, and personal-social interaction.

Summary: Music making and music therapy brings about some of the developmental changes necessary to achieving communicative interaction, and this is the basis of social interaction and independence. That these factors are pre-verbal and not lexically dependent, argues for the importance of music making in the treatment of developmentally delayed infants. Music therapy is a suitable integrative psychosocial treatment for children with developmental delay.

EP15.04 Participation in biobanks and research: Motivations and expectations of cancer patients

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Background: Biobanks have become strategic resources for biomedical and genetic research. Few empiric data exist describing patients' views.

Objectives: To investigate motivations and expectations of cancer patients when they are solicited to grant access to their own biological specimen for research projects.

Methods: The point of view of patients (n=19, aged 28-82) treated for colorectal cancer or leukaemia was explored by in depth interviews. Grounded theory was used to guide sampling, data collection and analysis.

Findings: All patients were willing to participate in biobanking and first motivated by a desire to help others and to contribute to advances in cancer research. Their attitudes were modulated by the context of the disease and by their previous attitudes towards donation in general, or in medical research context. Contribution to biobanks was further described as an act of solidarity and reciprocity, which makes patients as being part of a community (they benefit from past research and future patients may benefit from present research), but also as an individual experience with psychological issues (dimension of hope associated to research, of self rewarding and of personal empowerment).

Discussion: The results reported here are in support for a benevolence account of the act of contribution to biobanks, and urge on getting out of the cleavage between participants being truly altruistic or driven by personal gain. Knowledge of patients' perspective may guide the governance of biobanks, and facilitate the (re-)writing of information documents and consent forms, as well as establishment of institutional policies to collect these consents.

EP15.05 The course of human genetics for the students of the Family and Childhood Psychology Faculty of St.Petersburg University of Culture

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Hereditary and congenital disorders result in early and severe physical and mental disability. Interpersonal relations are tense and nervous in the families having children handicapped and psychological climate is far from positive one. More often a father can not bear it any longer and a marriage is broken down. These families stand in need of

psychological support. Psychologist has to understand and sense of these problems and difficulties. Knowledge of the milestones of human genetics gives good grounds for successful professional activity of the psychologists. In 2006 year a course of human genetics (28 hours) was included in the curriculum of the third year students of the Family and Childhood Psychology Faculty of St. Petersburg University of Culture. This course includes basic genetics, molecular and biochemical genetics, cytogenetics. The chromosomal and monogenic diseases are discussed using a lot of clinical cases. Roleplay in small group learning is used for teaching communication skills. The students have to create and analyze their own pedigrees. This task is one of the most important and difficult for them. All students studied with interest and showed enthusiasm. They confirmed that the knowledge of basic genetic problems were very important for their professional skill. In the context of the psychological problems of the families with children handicapped the psychologists will integrate the tools of genetics in their practice for the most successful prediction and prevention psychological conflicts.

EP15.06 An unexpected finding in the NF1 clinic

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This case report reflects on the genetic counselling implications of an unexpected new differential diagnosis in a 13 year old girl (LS) attending a NF1 Management clinic. It will discuss the implications of both breaking and receiving bad news in this context and the specific counselling considerations when the patient is an adolescent and accompanied by both her parents.

LS was referred by a paediatrician for diagnostic and management advice where NF1 was suspected but not confirmed by genetic testing. The patient had presented with neurofibromas of the tongue and thickened corneal nerves. Following clinical examination a diagnosis of NF1 was excluded but this was replaced by a new, potentially life threatening diagnosis requiring an urgent surgical assessment. How this information was communicated, the medical and psychosocial implications for LS and her family and the counselling implications will be discussed.

Kessler (1992) refers to the importance of transitions in genetic counselling consultations. In this case the shift in tone and nature of the information to be conveyed was traumatic for the family. With no prior preparation the medical consultant and genetic counsellor were faced with breaking bad news, outlining an urgent management plan and ensuring appropriate on-going support to the family. This paper will consider the counselling strategies adopted and the advantages and challenges of the co-counselling model in a complex and evolving situation.

EP15.07 Diamonds in the defects: logotherapy in medical genetics

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Introduction: Logotherapy (LT) is a form of psychotherapy based on the premise that we find healing through finding meaning. Meaning can be found in every situation in life, including the 'tragic triad' of pain/suffering, guilt and death. Logotherapy's founder, Viktor Frankl, was clear that it should be used in medical practice, but, to date, LT's main application has been in palliative care, psychiatry and the treatment of addictions. Presented here is my experience, as a clinical geneticist, with the use of LT in the field of medical genetics, a new application for this therapy.

Methods: During my year of advanced training in LT, I provided LT to four individuals, or their mothers, who either had birth defects or genetic disorders. All sessions were voice recorded, transcribed, and submitted for supervision. They were studied to identify the presenting logoproblem, achievements of each session, common emerging themes, and individual gains.

Results: Each participant will be briefly discussed in terms of background and presenting logoproblem. Common emerging themes will be highlighted, and individual meaning-making will be discussed.

Conclusion: All the participants found meaning in their suffering. Logotherapy is suitable for use in the context of medical genetics, and it

transcends cultural, educational and language barriers. Protracted counseling is not required to facilitate meaning-making..

EP15.08 Direct to consumer genetic testing : marketing strategies and ethical and societal issues.

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Direct to consumer genetic testing on Internet for various pathologies and behaviour traits appears as a marketing success. What kinds of commercial strategies use the companies offering these genetic tests and on what categories of social expectations do they play at the same time? Through a quantitative and qualitative analysis of the web sites offering such tests, it seems that these companies develop on the basis of the exploitation of a triple market: that of the „healthism“ (evolution of the mentalities by which health and hygiene are raised to the pantheon of the social values), that of the contemporary demands of the users of health systems to be able to become actual actors of the decisions concerning their health, and finally that of the bio-social relationship. Each of these three commercial strategies underpins various ethical and societal stakes that need to be explored in the context of this important and unprecedented development of this market of genetic tests.

EP15.09 Breast and ovarian cancer screening practices for non-carriers from BRCA1/2 mutation-positive families: Two-year follow-up of prospective cohorts from France and Quebec, Canada

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Background: We described and compared breast and ovarian screening practices in the 2-year period following test result disclosure in female non-carriers from BRCA1/2 mutation-positive families living in two countries, France and Quebec, Canada, which provide universal health care.

Methods: 402 (France n=293; Quebec n=109) unaffected female non-carriers from BRCA-proven mutation families provided information about the uptake of mammography, clinical breast examination, breast self-examination, and ovarian ultrasounds using self-administered questionnaires. The frequency of screening practices between study cohorts were compared using logistic regression.

Results: Annual mammography was conducted in 23% and 43% of French and Quebecer women participants <50 years of age, respectively (adjusted odds ratio [aOR] = 2.72; 95% CI, 1.08-6.81). In women ≥50 years of age, mammography was conducted in 49% and 65% of

French and Quebecer participants (aOR = 1.77; 95% CI, 0.07-4.51). Overall, 33% of French women and 39% of Quebecer women underwent at least one ovarian ultrasound during the 2-year period following BRCA1/2 test result with no significant difference between cohorts of women <50 years of age. Among older women, Quebecers reported more frequently than French women that they had undergone ultrasound once (aOR = 3.00; 95% CI, 1.02-8.83).

Conclusions: The frequency of cancer screening practices for female non-carriers from BRCA1/2 mutation-positive families in both France and Quebec exceeded those recommended for similarly aged women in the general population. Our findings highlight the need for clear-cut recommendations on the follow-up of women from BRCA1/2 families who are not themselves carriers of a BRCA1/2 mutation.

EP15.10 Producing genetics education resources for primary care practitioners is just the beginning: challenges in dissemination and engagement.

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Genetics in Family Medicine: The Australian Handbook for General Practitioners (GPs) (http://www.nhmrc.gov.au/your_health/egenetics/practitioners/gems.htm) was developed by the Genetics Education in Medicine consortium for the Australian Government in both print and on-line formats (June 2007). A summary sheet (*Genetics at a Glance (G@G)*) contained guidelines for practice from the 17 modules, contacts for genetics services throughout Australia and a web-link to the full resource which was also available in hardcopy (\$A40). Promotion included a small launch at a national GP conference and a mail-out of the G@G with the October 2007 edition of the journal Australian Family Physician to every registered medical practitioner. Two years later, 768 GPs working in an urban or a rural and remote area were approached to participate in a survey of the dissemination strategy and use and relevance of the resource to GPs' practice. While participation in the survey has been small (14% RR), early findings confirm the consortium's concerns that the dissemination strategy and lack of associated educational activities has resulted in extremely limited awareness of the resource: 17% remember receiving G@G and 6% can still locate it while only 4% and 1% have accessed the resource on-line or in print format, respectively. However, of those who opted into the learning activity for 2 CPD points, the vast majority found the information useful or very useful in completing the activity and the modules of information were rated highly as relevant to GP practice. Educational activities suggested by GPs to promote the resource will be presented.